Biosciences Directorate Overview and Highlights 2004

February 2005
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BIOSCIENCES DIRECTORATE
February 2005

The Biosciences Directorate seeks to contribute to the rapidly expanding science of biology in areas relevant to important challenges in national security, environmental sustainability, and human health.

To address these goals we have efforts focused on:

- the development of assays to detect and characterize the earliest molecular responses of humans to ionizing radiation and pathogen infections
- studies of pathogenicity mechanisms in microbial pathogens and key elements of the host response
- systems-level studies of the evolution and geochemical impact of the metabolic activity of environmental microbial communities
- comparative genomic and genetic studies to illuminate gene function and regulatory architecture
- mechanistic studies of DNA repair and of low dose radiation response
- the role of food-born mutagens in human cancer
- biotechnology development efforts in molecular recognition
- computational modeling at both molecular and cellular levels

These efforts are supported by core competencies in:

- microbial and mammalian genomics and genomic evolution — the functional analysis of genomes — the genes they encode, their evolution, their regulation, and their roles in living systems
- protein function and biochemistry — the structure, function, and interaction of proteins and other bio-molecules
- computational modeling at both the molecular and cellular level
- bioinformatics — databasing, networking, and analysis of molecular-biological data
- bio-instrumentation — the application of physical and engineering technologies to novel biological and biochemical measurements, laboratory automation, medical device development, and healthcare technologies

Our role within the broader Laboratory is to:

- Increase the contribution of the Laboratory to the accelerating scientific progress in the scientific comprehension of living systems
- Help the Laboratory contribute to the expanding role of the biological sciences in many areas of societal and national need
- Develop biological research that depends on, and directs the development of, technological capabilities throughout the laboratory, in physics, chemistry and materials science and computational science.

1.0 Research Areas and Achievements

Research Areas

The Biosciences Directorate includes five elements that reflect the new research areas of interest for the Program and reflect the scientific thrusts that comprise our strategic plan for bioscience at LLNL.

Biomedical Division
Our research in the Biomedical Division is broadly focused on determining the structure and function of proteins and nucleic acids and identifying how chemicals and radiation in our environment impact the genome, somatic cell function, and early embryonic development.

- A wide variety of computer modeling methods, ranging from homology-based protein structure prediction to highly computationally intensive first principles molecular dynamics, are used to predict the structures of the proteins and nucleic acids being examined, the interactions that occur between them, and the consequences that develop as a result of their damage.

- A variety of physical techniques, which include x-ray crystallography, nuclear magnetic resonance spectroscopy, mass spectrometry, various forms of spectroscopy (single molecule fluorescence, PIXE, XAFS, Raman) and microscopy (scanning probe, confocal), and several protein over-expression and purification technologies, are applied to the 1) structural and functional analysis of proteins and their complexes, 2) characterization of protein-nucleic acid interactions, 3) development of synthetic ligands that bind selectively to unique sites on the surfaces of proteins, and 4) the development of new metabolite-based methods for monitoring the functional state of an organism.

- Many studies focus on our developing an understanding of the mechanisms of radiation and chemical toxicities in cells so that we can reduce the individual and population health consequences of exposures through improved detection, prevention, and intervention. Biochemical, genetic, molecular and proteomic techniques are used to identify and characterize the molecular mechanisms that lead to DNA damage, as well as other cellular molecules, and those physiological and genetic mechanisms that determine cell survival, genomic integrity, and tissue homeostasis.

Recent Biomedical Division research has led to advances in understanding the consequences of DNA damage and the process of DNA repair, detecting genetically defective somatic and germinal cells, interrogating the genome-wide responses of cells exposed to toxicants, reducing the mutagenic effects in food preparation and understanding the mutagenic effects of potential anti-carcinogens, the development of new small molecule therapeutics for non-Hodgkins lymphoma, and the creation of synthetic high affinity ligands for pathogen detection.

Our research, which has benefited from the broad expertise provided by multidisciplinary teams, the availability of advanced biocomputing, state-of-the-art analytical instrumentation and the implementation of enabling genomic and proteomic technologies, is supported by funding from NIH, NSF, DOE, DHS, LDRD and Industry.

Biotechnology Division

This is a newly created division within the Biosciences Directorate, the focus of which is threefold:

- serve as a bi-directional conduit or gateway facilitating inter-directorate interaction/cooperation, e.g. with the Center for Accelerator Mass Spectrometry (Energy and Environment Directorate), the BioSecurity and Nanosciences Laboratory (Chemistry and Materials Science Directorate), and the National Cancer Institute's Early Detection Research Network,

- develop and offer state-of-the-art lab-wide service capabilities, e.g. LLNL Microarray Center and Biological NMR Center,

- contribute toward crafting a plan and to obtain funds from Department of Energy Genomics:Genomes to Life program to build "Facility 1" - Production and Characterization of Proteins and Molecular Tags - in joint effort with Sandia National Laboratory and others. Our approach will involve novel automation technologies, multiple protein synthesis capabilities (cytosolic and membrane proteins), protein specific molecular tag(s) or molecular recognition elements (MREs), sample archiving (cloned genes and proteins) and database management systems.

Defense Biology Division

Our work in the biodefense field focuses on providing both the basic bioscience and the tools necessary to render bioterrorism ineffective. Our work focuses on such diverse topics as detection of biowarfare threats, human and microbial forensics research and applications, and presymptomatic disease detection. We are building advanced detection systems to provide early warning, identify populations at risk and contaminated areas, and facilitate prompt treatment. We develop DNA signatures and biological forensics technologies to identify an infectious agent, its geographical origin, and/or the initial source of infection. Similar approaches are applied to human forensics, and are used in both law enforcement and intelligence-gathering activities. To carry out this work, our division has a strong partnership with the Nonproliferation, Arms Control, and International Security Directorate (NAI) here at LLNL, and is supported by a wide range of internal and external funding sources.

Genome Biology Division
Research in the Genome Biology Division is focused on generation of genomic resources and on developing tools and strategies for turning these basic data into information regarding genome evolution, gene regulation and biological function. The Division includes LLNL's members of the DOE Joint Genome Institute's (JGI) comparative mapping, DNA sequencing and annotation teams, and the I.M.A.G.E. Consortium, a central element of the international effort to document expressed sequences in a wide variety of eukaryotic genomes. Genome Biology also includes research groups using comparative genomics to identify critical DNA sequence elements in complex genomes and to trace conservation and change of these sequences in vertebrate evolution. Members are working specifically to (1) develop new computational tools for genome comparison and characterization of regulatory sequences and modules associated with co-regulated genes; (2) test and document the functions of predicted genes and regulatory elements in vitro and in vivo using model systems including mouse, chicken and the frog, *Xenopus tropicalis*; (3) track the biological impact of lineage-specific genes with special focus on those encoding zinc-finger transcription factors, the rapid evolution of which may play a central role in modeling and remodeling vertebrate regulatory networks; (4) unravel basic mechanisms involved in gene repression, including those underlying genomic imprinting; and (5) development of a unique set of mouse models for studying human gene function and inherited disease. Our goal is to provide genomic data, novel computational and experimental tools, and information regarding functions, regulation and evolution of vertebrate genes as a resource to the biological research community.

Microbial Systems Division
The Microbial Systems Division addresses a new area of growth for the Directorate tied closely to the goals of the DoE Office of Science Genomes-to-Life Program. A central focus for us in this effort will be to investigate the degree to which the net metabolic activity of a microbial community is predicable from the physical-chemical properties of the environment in which it operates. The core hypothesis under investigation is that microbial communities typically develop highly optimized solutions to the overall 'energy conversion' opportunities presented by the environment and that as a result these solutions are to a useful degree predicable from the properties of the environment.

With the support of the Laboratory, we are actively recruiting researchers and scientific leaders to develop the needed expertise in support of the DoE/OBER objectives.

Bioinformatics and Biostatistics Group
The Bioinformatics and Biostatistics group consists of computer scientists, bioinformaticians, statisticians, programming technicians, system administrators, and computer security and desktop support personnel. The group supports ongoing research and development within the Biosciences Directorate and the Joint Genome Institute (JGI) in Walnut Creek.

Group members are involved in nearly every project within the Biosciences Directorate and the JGI. Some examples include whole-genome analysis and annotation, microbial systems analysis, expression array research, comparative genomics, biostatistical consulting and methods development, high-throughput DNA sequencing, cDNA library analysis and maintenance, genome mapping, and administrative systems development and support.

Physical Biosciences Institute

The Physical Biosciences Institute (PBI), a LLNL University Relations Program Institute, officially opened in 2003. The goal of the PBI is to incubate new post-doctoral projects that use LLNL's advanced analytical capabilities to address questions in quantitative biology at the single-cell and single-molecule level. All of these projects involve collaborations with directorates outside the Biosciences Directorate and most of the projects involve collaborators at a University of California campus.

Note: in January 2005 the PBI moved from the Biosciences Directorate to the Chemistry and Materials Science Directorate.

Research Abstracts

The following abstracts highlight research being reviewed from 2004 by our scientific advisory board. Titles and authors are listed below for abstracts found on the following pages.

A unique mouse mutant resource for mapping essential genes and regulatory sequences
Colleen Elso

Genome mapping and annotation for the Joint Genome Institute (JGI)
Laurie Gordon

BMP-antagonists in skeletal and limb development
Gabriela Loots

Characterizing evolutionary conserved elements in the frog *X. tropicalis*
Gabriela Loots

I.M.A.G.E. Consortium update
Christa Prange

Evolution and regulatory function of human zinc finger transcription factor genes
Lisa Stubbs

Functional Annotation of genes and regulatory sequences on human chromosome 19
Lisa Stubbs

Pathomics: Molecular Signatures of Host Response for Detection of Biothreat Agent Exposure
Ken Turteltaub

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A unique mouse mutant resource for mapping essential genes and regulatory sequences

Colleen Elso, Angela Tarver, Stephanie Morrison, Laura Chittenden, and Lisa Stubbs

The mechanisms and rates by which exposure to radiation and certain types of chemical agents induce human mutations have been a focus of DOE research for many decades. This project capitalizes upon DOE's long-term investment in mutagenesis, by completing the genetic and molecular characterization of a collection of mutant animals that arose in mouse genetics studies at the Oak Ridge National Laboratory (ORNL). Our studies focus specifically on animals carrying reciprocal translocations associated with visible phenotypes caused by disruption of genes at the breakpoint sites. In previous studies we selected 29 mutant strains in phenotypic screens and characterized the behavioral, developmental, or fitness defects these animals display. Since translocations visibly disrupt chromosome structure, they can be mapped efficiently to small physical intervals without genetic crosses, using fluorescent in situ hybridization (FISH). With the availability of mouse genome sequence, we have mapped breakpoints of 13 mutations to single bacterial artificial chromosome (BAC) clones and sequenced 9 breakpoint sites in 5 of the mutant mouse strains in the past 1.5 years. From these data we have identified genes that are responsible for mutant phenotypes in 9 mutant strains and candidate genes for an additional 4 mutants. Mapping is now underway for the remaining mutant stocks, and we expect to complete BAC mapping by the end of this year.

Mouse translocations are very rare, and few have been characterized on the molecular level. Our sequencing data show clearly that translocations produced by a wide variety of treatments, including radiation and several different chemicals, arose through a mechanism involving non-homologous end joining (NHEJ). This mechanism has also been associated with spontaneous translocations in humans, but has not until now been linked to lesions induced by specific mutagens. The breakpoints carry remarkably "clean" molecular breaks that are focused tightly at chromosome junctions and leave surrounding DNA intact. Eight mutations break within genes, while four of the mapped mutations break outside of the affected transcription units and appear to disrupt or detach critical regulatory elements. One mutation, 14Gso, breaks within the Igf2 imprinted domain and appears to disrupt the parent-of-origin specific expression of nearby genes. Most of the genes disrupted by these mutations are exceptionally large, and present an especially large "target" for gene disruption. Six mutations represent new alleles of existing spontaneous or targeted mouse mutations and provide new information regarding the function or regulation of those genes. For example, our recently published studies have linked mutations in the imprinted gene, Kcnq1, to a heightened susceptibility to gastric infection and stomach cancer, a phenotype that was not observed in knockout mice. The 12Gso mouse is a null mutation of Tbx18, a gene involved in somite development, but the mutation occurs downstream of transcribed sequences and appears to separate Tbx18 from essential regulatory elements (Figure). We are now focusing on completing studies of novel gene mutations associated with developmental defects, neurological abnormalities, obesity and cancer for publication, and proceeding to map mutations associated with the remaining mutant stocks. In future studies we will focus

on developing methods to characterize the novel regulatory mutations and study the impact of translocations on regulation of imprinting.

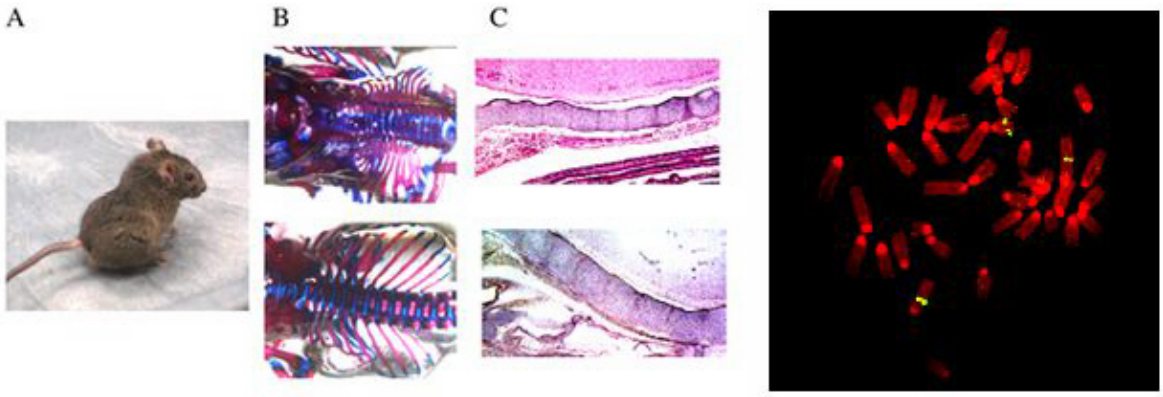

Figure legend. The 12Gso mouse. Panel A: a 3 week old animal with wavy tail and shortened back. Panel B: histological stain of bone (red) and cartilage shows the disorganized, compressed ribs and vertebrae in 12Gso (top) vs normal littermate (bottom). Panel C: H&E stain of a sectioned prevertebral column from a 17.5 dpc 12Gso mutant (top) and normal (bottom) embryo indicates abnormal development of somites. At far right, a FISH image showing results of hybridizing a breakpoint-spanning BAC clone to metaphase chromosomes of a 12Gso/+ mouse. The "split" signal this BAC produces indicates that sequences within the clone are separated onto separate chromosomes in the mutant mouse. This mutation breaks approximately 40 kb downstream of a somite-specific gene, Tbx18, and disrupts the gene's expression, presumably by detaching the coding sequences from a critical regulatory sequence.

Genome Mapping and Annotation for the Joint Genome Institute (JGI)

Laurie Gordon[1,2], Paul Butler[1], Mari Christensen[1], Elizabeth Fields[1], Richard Nandkeshwar[1], Mary Tran-Gyamfi[1], Ivan Ovcharenko[1], Susan Lucas[1,2], and Lisa Stubbs[1]

The Livermore-based JGI Genome Mapping Team performs targeted genome mapping to identify clones of interest for sequencing at the Production Genomics Facility (PGF) in Walnut Creek. This group specializes in cross-species comparative mapping, designing DNA probes across species boundaries, performing high density ^{32}P filter hybridizations and constructing restriction maps from which to identify efficient sequencing tiling paths. This methodology complements prevailing whole genome shotgun sequencing strategies (wgs) to improve assembly quality, and can be very useful where conventional wgs fails.

Completed projects include:

- Map closure, sequencing tiling path selection and sequence annotation of human chromosome 19 (HSA19). A JGI publication detailing the sequence and biology of HSA19 completes this effort: Grimwood, et al. (2004) *Nature* 428:529-35.
- Map closure of HSA5 (LBNL) and HSA16 (LANL) to support timely completion of DOE's sequencing commitments. See JGI publications: Schmutz, et al. (2004) *Nature* 431:238-274 and Martin, et al. (2004) *Nature* 432:988-994.
- Comparative mapping of mouse genomic regions homologous to HSA19. This work was previously reported: *Genomics* 74:129-141 and *Science* 293:104-111.

Current projects include:

- Targeted mapping of *Xenopus tropicalis* (frog) genomic regions homologous to 30 Mb of the human genome as specified by the NIH ENCODE project. 236 clones (14.6 Mb, 246 orthologs) have been submitted for sequencing; a second round of hybridizations is underway to close map contigs. We also identified 109 (9.1 Mb) clones for individual frog genes of interest to JGI collaborators.
- Identification of fosmid clones containing genes for *Phakopsora pachyrizhii* (soybean rust), a fungus with potentially devastating agricultural consequences. Due to high repeat content this organism could not be sequenced with conventional wgs. We have developed probes and conducted hybridizations for the first 50 (of 100) genes; 9 clones have been fully QC'd and submitted for sequencing.
- Targeted mapping and sequencing of *Gallus gallus* (chicken) genomic regions homologous to HSA19 resulted in assembly and annotation of 3 contigs, GGA28 (1.1 and 3.6 Mb) and GGA11 (3.7 Mb). These data correct order and orientation errors relative to the published chicken wgs (*Nature* 452:695-716), and properly locate an additional >400 kb. A catalog has been prepared detailing gene content and characterizing homology rearrangements. The data demonstrate a spectrum of protein conservation and divergence; synteny assessment enhances orthology assignment; duplications are a common feature at homology breaks. Manuscript in preparation.

Team personnel also contribute annotation expertise in support of other Genome Biology Division initiatives, including functional annotation of HSA19 and ZNF gene families.

[1]Genome Biology Division, Biosciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA, and [2] Production Genomics Facility, Joint Genome Institute, Walnut Creek, CA.

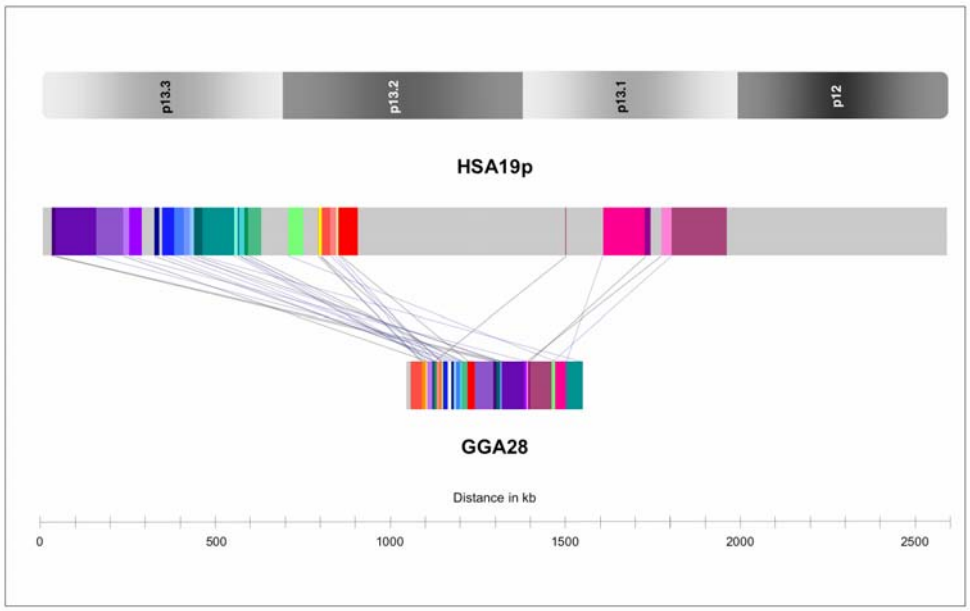

Figure 1. Chicken chromosome 28 (GGA28) contains 30 major homology segments syntenically related to the short arm of human chromosome19 (HSA19p). Due to lower repeat content in chicken the human:chicken genomic size ratio averages 2.25:1. Segments connected by black lines are oriented in the forward direction, gray lines indicate segments in reverse orientation.

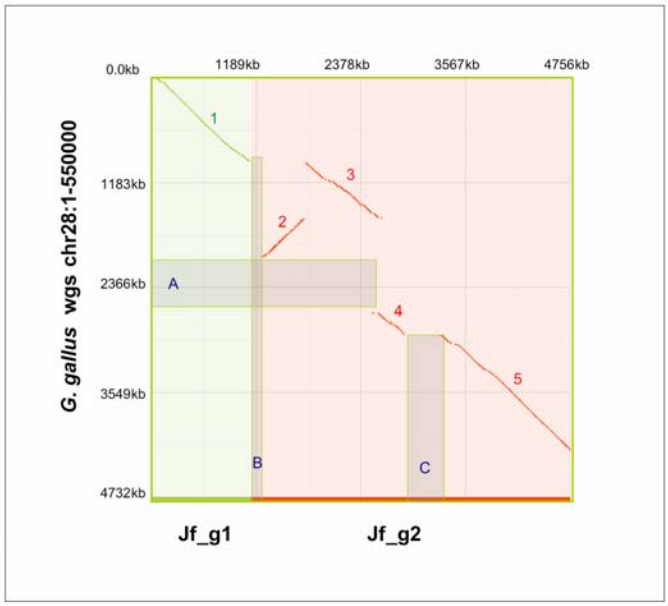

Figure 2. Map-driven assembly with clone-based, high quality draft sequence improves coverage, as well as order and orientation of supercontigs, relative to the whole genome shotgun assembly (*Nature* 452:695-716, 2004). For instance, orientation of segment 2 has been corrected, and segments 3 and 2 have been correctly ordered. Gap A is an HSA18-related supercontig incorrectly assigned to chr28 in the wgs; gaps B & C represent additional HSA19-related sequence that has been correctly localized in our assembly.

BMP-antagonists in skeletal and limb development

Nicole Collette[1], Jessie Chang[1], Richard M. Harland[2] and Gabriela G. Loots[1]

The goal of our research is to understand the molecular mechanisms by which two related progressive bone disorders: Sclerosteosis (MIM 269500) and van Buchem disease (VB) (MIM 239100) lead to pronounced radiological bone alterations (jaw; long bones; gigantism) and hand abnormalities (syndactyly). Both these disorders map to the same locus on chromosome 17 (17q12-q21) in the vicinity of the BMP-antagonist Sclerostin (Sost), and Sclerosteosis patients carry Sost coding mutations. VB patients carry a homozygous 52kb noncoding deletion downstream of the gene mutated in Sclerosteosis. By combining in vitro bacterial artificial chromosome (BAC) recombination techniques and mouse transgenic technologies we have shown that this 52kb noncoding deletion alters the expression pattern of human Sost in transgenic mice. Therefore, we hypothesize that VB is caused by the removal of essential Sost-specific transcription regulatory elements, and therefore is allelic to Sclerosteosis. Our main goals are to understand the genetics of VB and the transcriptional regulation of this negative regulator of bone production. In addition, transgenic mice expressing human Sost exhibit severe limb abnormalities and osteopenia, in a dosage dependent manner. These studies suggest that Sost skeletal and embryonic expressions are due to separate enhancer elements. We hypothesize that this BMP-antagonist plays critical roles during embryonic limb development and adult bone homeostasis, and its levels of tissue and stage-specific gene expression dramatically impact the patterning and integrity of the vertebrate skeletal system. Our research focuses on understanding the genetic and physiological properties of Sost in several vertebrate systems by elucidating its embryonic function, complex transcriptional regulation, its involvement in congenital skeletal disorders and bone remodeling.

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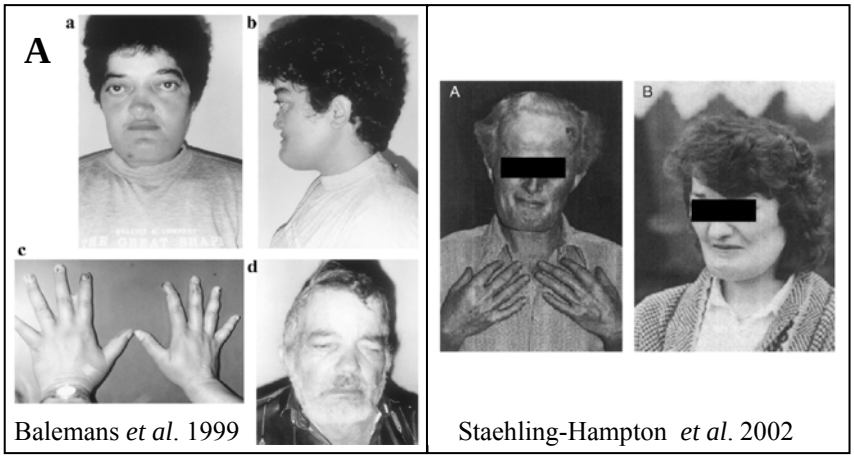

Figure 1. Sclerosteosis (A) and Van Buchem (B) patients exhibit highly similar phenotypes with the exception of syndactyly of the digits, which is characteristic of sclerosteosis.

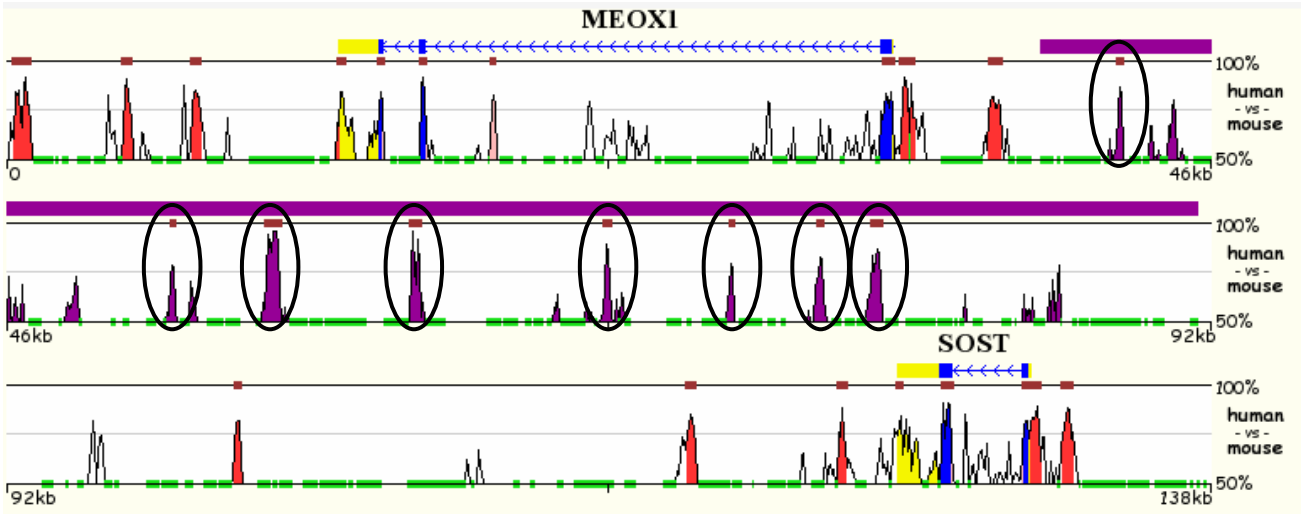

Figure 2. 138kb human and mouse sequence alignment for the Sost gene locus from human chromosome 17 including the Sost and Meox1 transcripts as well as the noncoding region deleted in van Buchem patients. The human sequence was used as reference, with distance in kb on the x-axis, and percent identity between mice and humans on the y-axis. Red boxes correspond to regions of high conservation (≥200 bp/≥80% ID). Exons are in blue, untranslated regions are in yellow, intragenic noncoding elements are in red and intronic conserved elements are in pink. DNA peaks present in the van Buchem deletion region are colored purple, and the highly conserved elements are circled. This alignment was generated using the zPicture tool (http://zpicture.dcode.org/).

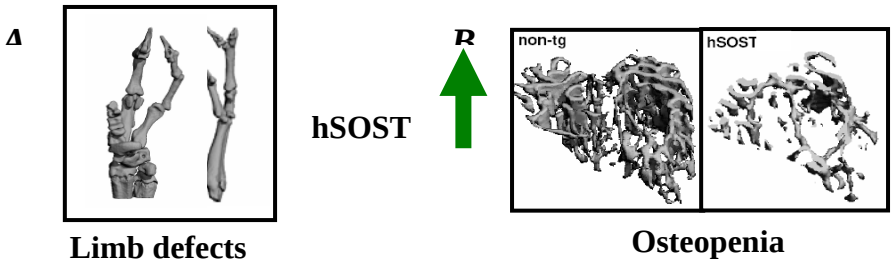

Figure 3. High levels of human Sost expressed in transgenic mice result in limb defects (A) and osteopenia (B).

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Characterizing evolutionary conserved elements in the frog *X. tropicalis*

Ivan Ovcharenko[1], Jessie Chang[1], Nicole Collette[1], Praveen Babu[1], Mustafa K. Khokha[2], Richard M. Harland[2] and Gabriela G. Loots[1]

We have coupled comparative genomic analysis with high throughput experimental approaches in the frog, Xenopus tropicalis, to identify and determine the biological function of evolutionarily conserved human sequence elements. Early vertebrate development proceeds along remarkably similar pathways in frogs and mammals, so that most events monitored in a developing frog can be extrapolated faithfully to humans. The high throughput approaches of genomic research have been relatively difficult to apply to mammalian embryology due to their small size, limited numbers of embryos, and internal development. In contrast amphibian embryos are abundant, large and free-living. Early events during vertebrate development are critical for establishing the cell-signaling pathways and cell fates that specify the highly specialized adult morphology. The majority of developmental genes identified in frogs have been found to have highly conserved orthologs in mouse and human genomes. We have aligned the human genome with 2 rodent, 3 fishes, the chicken and the frog genomic draft sequences, in order to identify and catalog all the transcripts and conserved noncoding elements shared by all these vertebrates. Using well-established manipulations techniques in X. tropicalis we are determining the expression patterns of deeply conserved novel genes by in situ hybridization and have designed a high-throughput pipeline to determine the function of these genes by mRNA overexpression and morpholino knock-down analysis. In parallel we are developing transgenic technologies to identify embryonic enhancers in the frog with the goal of characterizing regulatory networks of developmentally expressed genes. Through comparative genomics and development we are extrapolating the information derived from amphibians to humans to learn more about evolutionary conserved functions in vertebrate genomes.

[1]Genome Biology Division, Biosciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA, USA and [2]Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA.

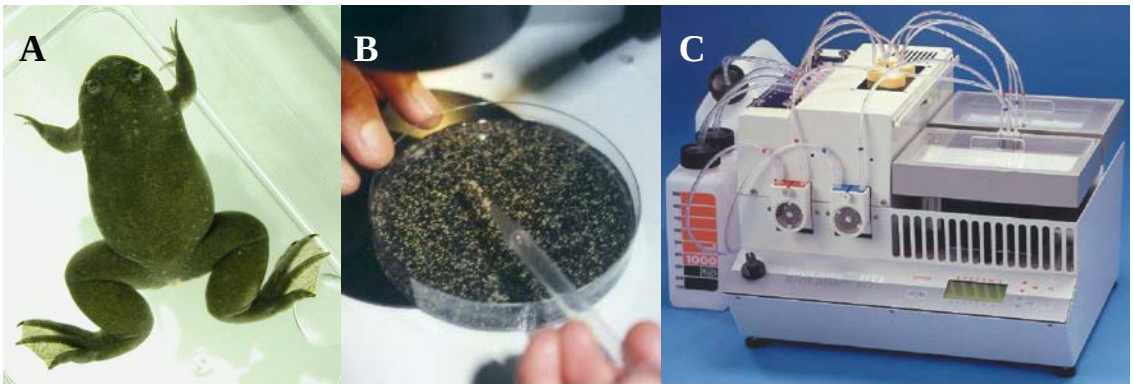

Figure 1. (A). Adult *Xenopus tropicalis* frog. (B). In vitro fertilization of eggs obtained from one hormone induced female frog. (C). In situ expression patterns are determined using automation with the in situ hybridization robot.

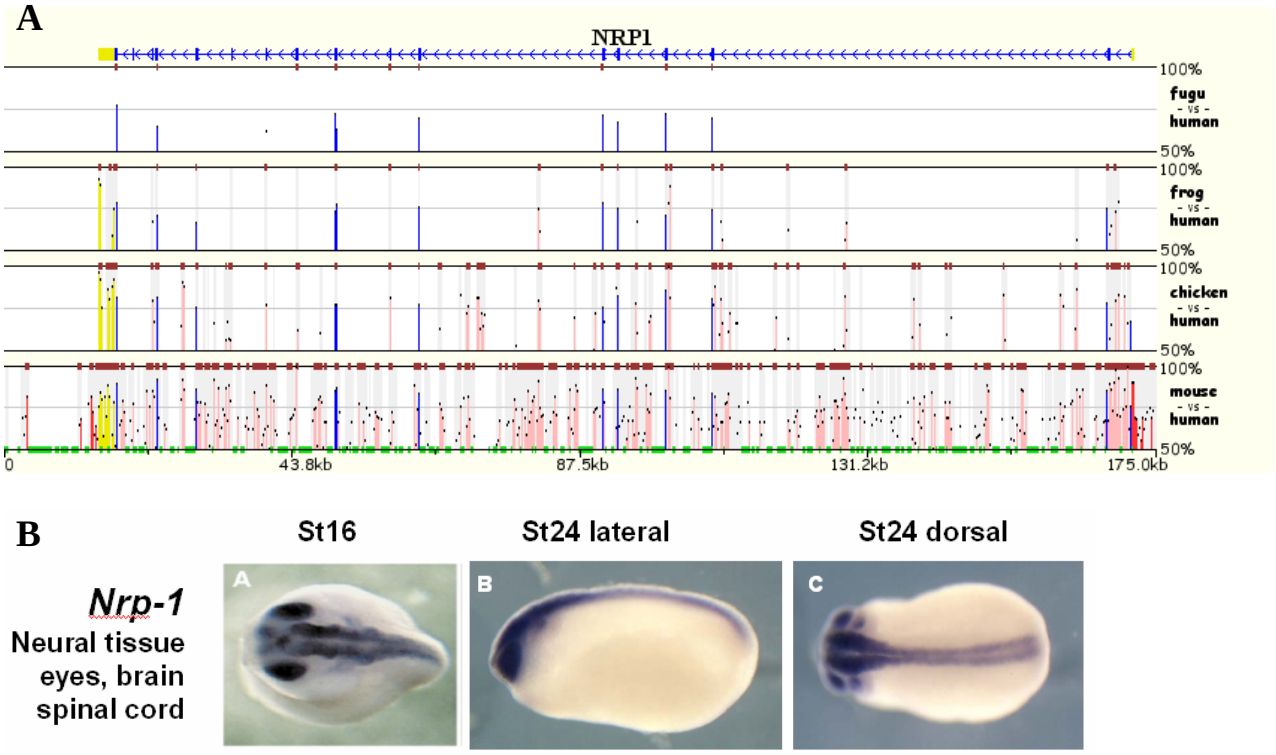

Figure 2. Frog genome has been aligned to the human genome in the ECR Browser (http://ecrbrowser.dcode.org/). Using various computational filters we identify highly conserved genes and prioritize them for expression analysis. Here NRP-1, is a highly conserved gene identified in our analysis (A), and is expressed during gastrulation (B-A) and neurulation (B-B,C) as determined by in situ analysis (B).

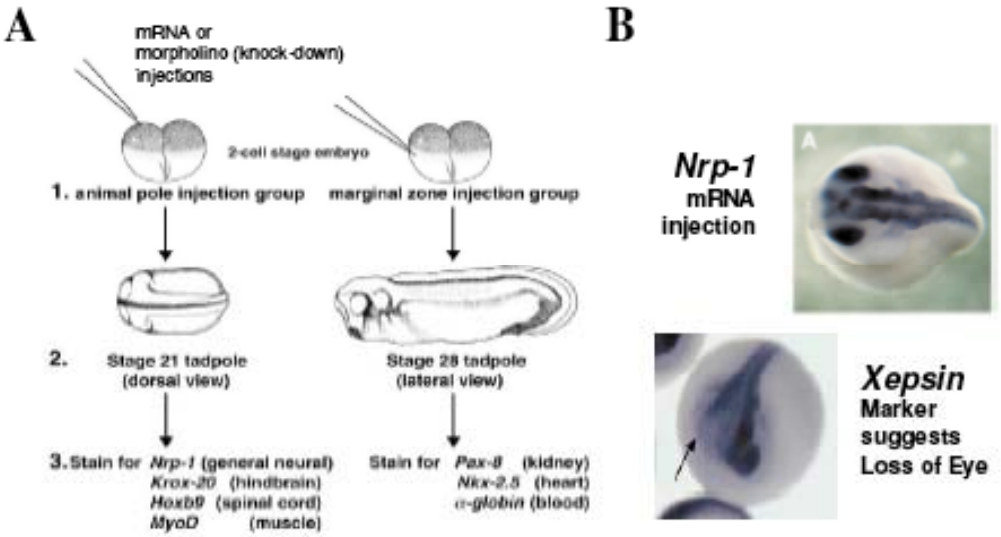

Figure 3. To determine the function of novel genes, we inject mRNA or morpholinos into 2-cell stage embryo and observe morphological changes (A). If no obvious morphological changes are observed, molecular probes are used to examine subtle phenotypes, in this situation overexpression of Nrp-1 results in loss of Xepsin which indicates early loss of eye structures (B).

I.M.A.G.E. Consortium update

Christa Prange, Mike Firpo[1], Amber Marsh[1], Liz Wilgus[1], Nicole Shapiro, Kirsten Schreiber

The primary goal of the I.M.A.G.E Consortium is to provide an arrayed cDNA resource useful for studying genes and proteins, as well as accompanying data management tools. The I.M.A.G.E. Consortium produces the largest publicly available collection of cDNAs; currently encompassing over eight million clones from eight species. These clones are arrayed at LLNL and sequenced at various institutes, and the resulting sequences are immediately deposited into Genbank. The clones themselves are made available royalty-free through a network of five distributors worldwide.

The I.M.A.G.E. Consortium is currently focused on generating arrayed cDNAs from full-length-enriched libraries. There are several species being targeted as part of the Mammalian Gene Collection (MGC) project, an NIH-sponsored effort to generate full-length cDNA resources from human, mouse and rat (http://mgc.nci.nih.gov). Accompanying these mammalian projects are efforts in *Xenopus* (both *laevis* and *tropicalis*), zebrafish and cow. Both single-pass and full-insert sequences are generated from full-length enriched cDNA libraries. As of February 2005, over 42K distinct full-length genes have been sequenced to high quality from the species mentioned above (with an emphasis on human and mouse), with additional clones in the pipeline.

To work toward the MGC goal of generating a full-length, sequence-verified clone for each gene, the strategy now includes targeting missing genes in a directed manner, producing clones using RT-PCR products made from gene-specific primers. In general, the types of genes missing from the collection include well-characterized genes that are long and/or expressed at very low levels and also gene models based solely on evidence from gene prediction programs. Future MGC goals include converting the current full-length cDNA collections into full open reading frame clones (i.e. containing no 5' or 3' UTR) based in a recombinational cloning system. This would allow for direct use of MGC clones for large-scale expression and proteomics applications.

Genome Biology Division, Biosciences Directorate and [1]Engineering, Environment, Biology and Institutional Computing Division, Computations; LLNL

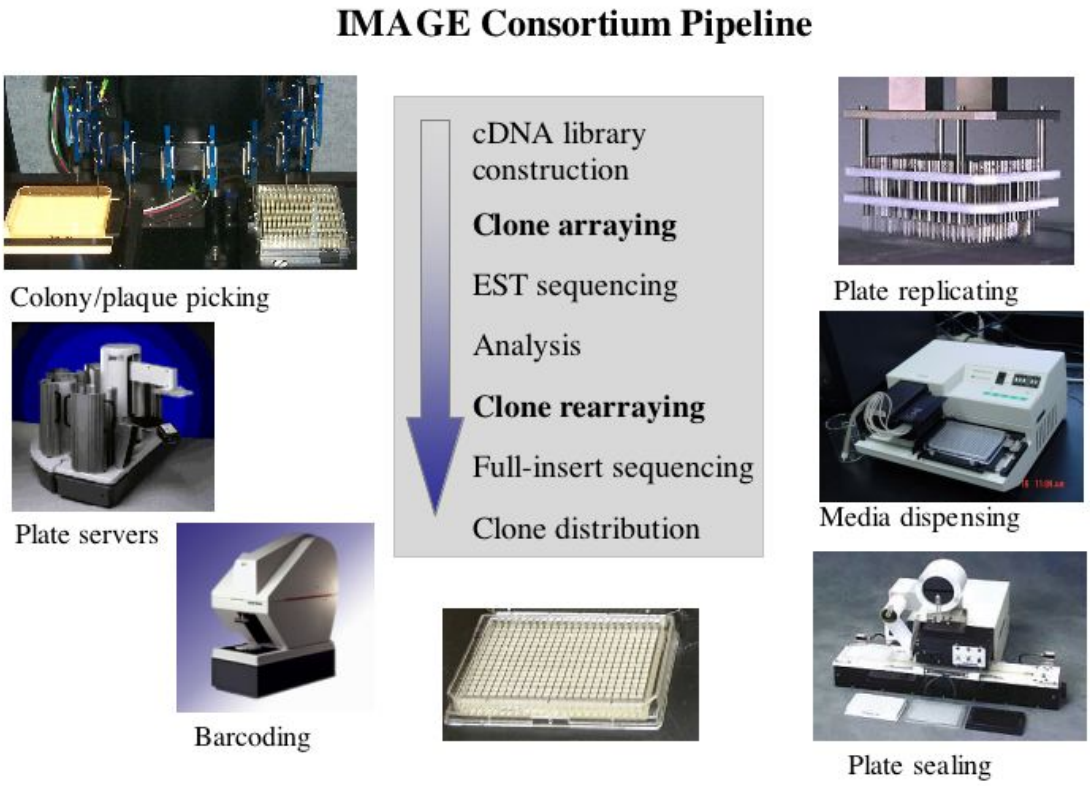

Figure legend. The I.M.A.G.E. Consortium pipeline depends on many institutions to provide a high-quality resource. cDNA libraries are constructed by multiple centers to ensure that the most successful methods are used to provide libraries highly enriched for full-length clones. Clones are arrayed at LLNL into 384-well plates using a Norgren Systems picker, and duplicated using a Genomic Solutions replicating robot. The copied plates are sent to several large-scale sequencing centers who produce single-pass reads at the 5' end of the cDNA for immediate submission to Genbank. The sequences are analyzed at the National Center for Biotechnology Information (NCBI) to predict whether or not the clone contains the full coding sequence. Clones which are predicted to be full-length and not already present in the MGC collection are rearrayed at LLNL from the original source plates and send to various sequencing centers for full-insert sequencing to a very high standard (<1 error in 50,000 bp). These clones are also sent to five clone distributors to make the clones available worldwide.

Evolution and regulatory function of human zinc finger transcription factor genes

Lisa Stubbs, Aaron Hamilton, Stuart Huntley, Alice Yamada, Dan Baggott, Laurie Gordon, Mary Tran-Gyamfi, Kathryn Segalle, Meiye Wu, [1]Bill Bosl, [2]Jeffrey B.-H. Tok, and [2]Julio Camarero

The development of accurate models for gene regulation in complex genomes will depend on a full understanding of regulatory DNA sequences and the transcription factor (TFs) proteins that bind to and control their activities. However, binding specificities are known for only a small fraction of TFs in any living species, including less than 20% of the predicted TF proteins in the human genome. Zinc-finger (ZNF) proteins comprise one of the largest classes of TF proteins in metazoan species; more than 1000 ZNF proteins exist in the human genome and approximately half belong to a single subfamily, in which a strong repressor motif, called the Kruppel-associated box (KRAB) is attached to the DNA binding ZNF domain of the protein. KRAB-ZNF proteins are unique to tetrapod species, expanding from a small family in amphibia and birds to approximately 500 members in mammals. This rapid expansion has continued throughout mammalian evolution, mostly through tandem in situ duplications of ancestral genes. KRAB-ZNF gene duplication has continued independently in different vertebrate lineages, yielding families of related but structurally distinct proteins in every mammalian species. Our studies have shown that newly duplicated proteins diverge rapidly once they are created, through processes that are likely to select new DNA binding specificities at remarkable evolutionary rates.

Since KRAB-ZNF proteins encode transcription factors, their rapid evolution and lineage-specific divergence is likely to have had significant impact on vertebrate gene-regulatory networks. However, gene targets and binding sites are known for only a handful of KRAB-ZNF TFs. In fact, because of their recent duplication, structural similarity, and the highly specific expression patterns of many KRAB-ZNF genes, a large fraction of these protein coding sequences do not correspond to “known genes”. We have developed a complete catalog of human KRAB-ZNF proteins, including 150 novel genes, and have generated complete gene models for many genes that are incompletely or wrongly annotated in public databases. Included in this collection of genes are more than 150 primate-specific loci, with at least 50 genes that appear to have arisen by duplication within the past 1-2 million years. Our studies indicate that clustered ZNF genes appear to be both gained and lost at high frequency, driven at least in part by illegitimate recombination, and suggest that gene copy number and structure may vary significantly even within species . We are presently focused on developing a set of genetic and biochemical strategies for high throughput discovery of the regulatory targets of these rapidly evolving TF proteins. These combined methods will permit us to determine the pathways in which both deeply conserved and species-specific transcription factors participate and understand the role of the remarkable evolutionary volatility of this large gene family on speciation, intraspecies diversity, and individual aspects of biology.

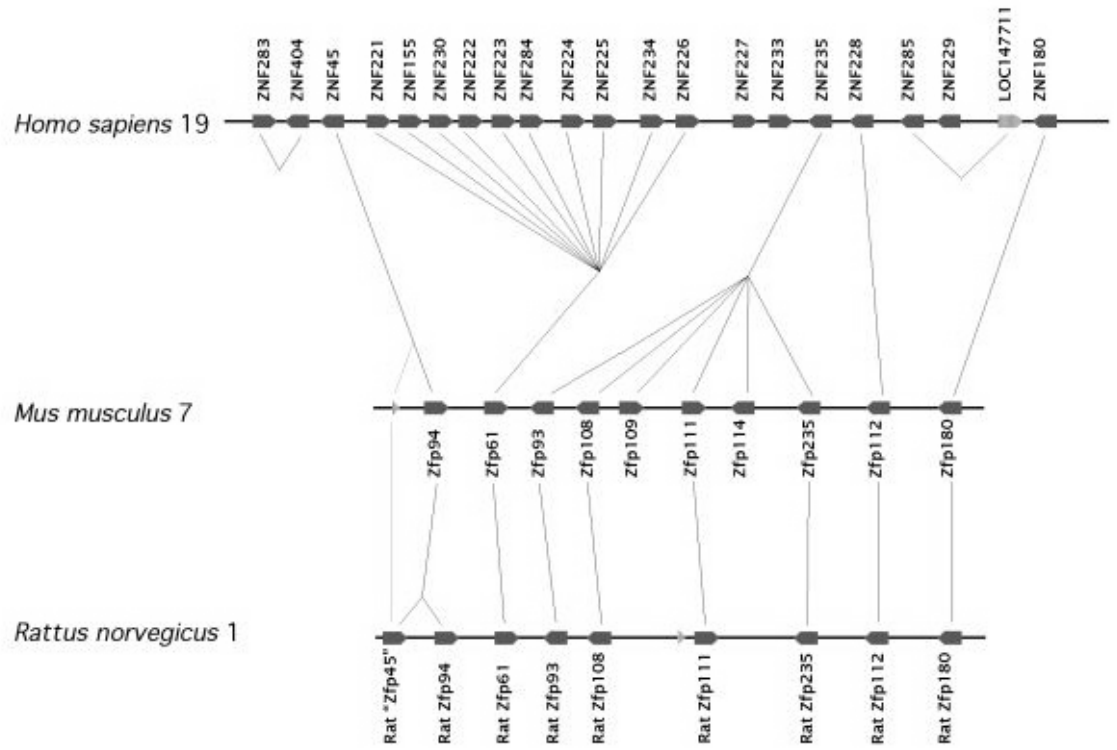

Figure Legend. Most KRAB-ZNF transcription factor genes reside in clusters that arose from tandem in situ duplications of ancestral genes, and the majority of human clusters are represented by homologous clusters in the genomes of other mammals. However, since gene duplication has continued independently since the mammalian radiation, each species carries different numbers and types of ZNF genes. The figure above summarizes relationships between genes in related clusters located in syntenically homologous regions of human chromosome 19, mouse chromosome 7, and rat chromosome 1, respectively. Vertical lines connecting clusters in different species summarizes the evolutionary relationships between resident genes. One-to-one orthology relationships are rare in ZNF gene clusters, due to the ongoing, independent duplication and loss of genes. Our studies have shown that KRAB-ZNF genes diverge quickly once they are duplicated, changing structure in ways that are likely to produce new proteins with distinct DNA binding specificities. This pattern of divergence suggests that mammalian gene regulatory networks are also evolving, with novel transcription factor proteins arising to repress target genes in a species-specific pattern.

Genome Biology Division, Biosciences Directorate; [1]Center for Advanced Scientific Computing, Computations; and [2]Chemistry and Materials Sciences Directorate, LLNL

Functional Annotation of genes and regulatory sequences on human chromosome 19

Lisa Stubbs, Laurie Gordon, Xiaochen Lu, Shan Yang, Jutta Kollet, Mary Tran-Gyamfi, Sha Coleman, Thomas Gulham, Eddie Wehri, Deepa Murugesh, Dan Baggott, Gawain Lavers, [1]Ivan Ovcharenko

Although the human genome sequence is finished and first-pass annotation of gene sequences has been completed, a considerable amount of work remains before the sequence can be used effectively to solve basic/mechanistic and health-related problems in biology. This project aims to lay the groundwork for functional studies in one human chromosome, HSA19, comprising the most gene-rich territory of the human genome and one of three human chromosomes fully sequenced by DOE-JGI teams. Members of our group participated in finishing and preliminary gene annotation of HSA19 and we are continuing to update and experimentally validate transcript models for the >1450 HSA19 genes. In addition, we are using comparative genomics approaches to predict non-coding regulatory elements in HSA19 sequence and assaying their functions using reporter assays in cultured cell lines. More recently, we have focused on experimentally testing the efficacy of different methods of transcription factor binding site (TFBS) prediction, using chromatin immunoprecipitation (ChIP) and related techniques. We have focused on use of prediction tools that rely on interspecies conservation, and have explored the value of pairwise and multi-species sequence alignments and other factors in the prediction of functional TFBS. To add depth to these studies, we collaborated with JGI teams to derive high-quality, clone-based sequence from HSA19-related chicken genomic regions. Gene-rich HSA19 regions were particularly recalcitrant to whole genome shotgun sequencing methods, and our sequence therefore provided an important contribution to the international efforts to sequence the chicken genome. Also in support of annotation efforts, we developed several new tools for conserved sequence identification and analysis, laying the groundwork for a comprehensive map of active promoters, enhancers, and TFBS throughout this gene-rich chromosome and elsewhere in vertebrate genomes. To complement gene and regulatory element annotation studies, we are developing a comprehensive map of expression for HSA19 gene homologs using in situ hybridization (ISH) in sectioned embryonic and neonatal mice. These data provide a "whole body scan" of gene expression that can be resolved to the level of single cells at different times in development (Figure). We have added data from sectioned chicken embryos for evolutionary comparisons of selected genes, and adult human tissue arrays to determine expression patterns for primate-specific gene duplicates. To date, we have focused primarily on perfecting methods of efficient probe design, tissue preparation, hybridization/staining and data capture and have succeeded in developing robust, fast and economical experimental protocols.

As a critical step in making high throughput ISH analysis a reality, our current challenge is to develop informatics tools to support data tracking, image archiving, annotation of images, and public query and display. We are also working to update HSA19 gene and transcript models, and develop a catalog of HSA19 regulatory sequences that are active in different living tissues using a whole chromosome DNA chip. We are also continuing development and experimental testing of comparative analysis and TFBS prediction tools, and beginning plans for database tools to link these different types of functional data into a publicly available resource for HSA19 gene structure, regulation and function.

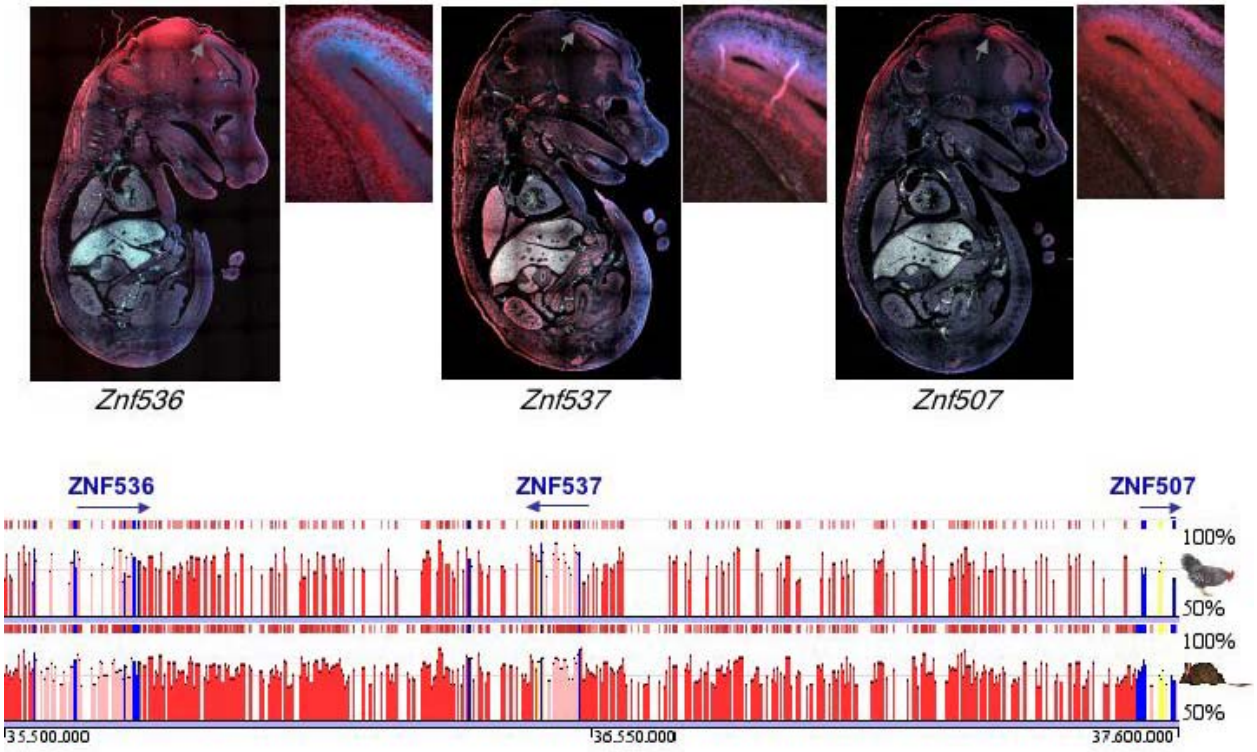

Figure Legend. Expression patterns of the homologs of three neighboring HSA19 genes, ZNF536, ZNF537, and ZNF507, in sectioned mouse embryos at day 14.5 in development (top panels). The three unrelated zinc finger genes are distributed throughout a 2 Mb region comprising the only "gene desert" interval in the otherwise very gene-dense chromosome. Despite the low density of coding sequences, this region is packed with sequence elements that are deeply conserved in vertebrates (bottom panel, showing a display from the ECR browser tool developed as part of this project). Our recent studies confirm that many of these conserved sequence elements function as tissue-specific enhancers and actively bind transcription factor proteins of specific types. In each embryo section, gene expression is detected by probes labeled with red fluorescent dye; background tissues are counterstained in blue to highlight anatomical detail and the outline of individual cells. All three genes are expressed highly in the nervous system, but different cellular distributions (i.e. the developing hippocampus, shown in insets at right of each section). Like its Drosophila homolog, teashirt, ZNF537 is also expressed in trunk structures including gut, kidney, and lung. The expression patterns of all three genes change dynamically over the course of development (not shown). In the ECR panel below, conserved sequences (or ECRs) detected in alignments between the 2Mb human region and related regions in mouse (lower layer) and chicken (upper layer) are displayed as colored peaks along the human map. Blue peaks correspond to coding exons, while pink and red peaks represent intronic and intergenic ECRs, respectively.

Genome Biology Division, Biosciences Directorate and [1]Engineering, Environment, Biology and Institutional Computing Division, Computations; LLNL

Pathomics: Molecular Signatures of Host Response for Detection of Biothreat Agent Exposure

Ken Turteltaub, Ted Rigl, Rich Langlois, Michael S. Ascher, Kevin McLoughlin, David Nelson, Lyndsay Radnedge and Kodumundi Venkateswaran

Pathomics is a feasibility effort focused on developing a comprehensive strategy to understand an individual's response to infectious disease agents at the molecular level, particularly those that represent a threat from bioterrorism. Its overall goal is to discover molecules (biomarkers) that are indicative of an infectious disease process and develop molecular signature-based diagnostics to detect a developing infection as early as possible following exposure. Initial studies have focused on evaluating analytical techniques for high-throughput molecular discovery. Using a mouse model of cowpox virus infection, and the methods mass spectrometry, 2-D gel electrophoresis, and mRNA and antibody-based panels, we conducted studies to determine if molecular signatures of developing infection could be detected and how early in the process changes could be found. We are completing studies to assess variation between apparently healthy animals and humans to understand background and what levels of signal change are necessary to detect infection. We are conducting pilot studies to show that we can distinguish sick from apparently healthy people, to distinguish a bacterial from a viral infection and to test how early our methods can pick up a developing infection. By the end of the project, we hope to demonstrate that blood-borne signatures develop prior to overt illness. Our goal for this project is to prove the feasibility of this approach and propose a diagnostic system for population-based monitoring and triage of potentially exposed individuals during a large-scale epidemic such as might arise from a bioterrorism attack.

Mission Relevance
This research focuses on gaps in our ability to detect and mitigate the effects of infectious disease, whether natural or from acts of bioterrorism. It is particularly relevant to assessing how to detect new emerging diseases and detect genetic modification of pathogens based on the response of the host.

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Significant Achievements

Much of the research within the Biosciences Directorate crosses organizational boundaries. We have used general science topics as well as scientific groups as subdivisions to highlight the variety of research achievements accomplished during 2004.

Biomedical Division

- Determined the NMR solution structure of a tobacco-specific carcinogen covalently attached to the O6 position of guanine (O6-[4-oxo-4-(3-pyridyl)butyl]guanine adduct) positioned opposite dT in an 11mer DNA duplex.

- Developed the first prototype synthetic high affinity ligand that binds selectively to human lymphoma cells containing HLA-DR10 for use in radio-immunotherapy.

- Created and characterized two knock-out cell lines of proteins involved in DNA repair and the disease Fanconi Anemia.

- Described and presented a new mechanistic model for the role of Fanconi anemia proteins in DNA replication and cancer progression.

- Developed novel data-based driven methods to ultra-fast structural correlations in proteins.

- Designed and demonstrated first Brownian dynamics simulation of dynamic supercoiling in long DNA chains.

- Developed novel analyses protocols based on artificial intelligence methods to identify a transition in gene-transcript expression between low and high ionizing doses.

- Identified potential biomarkers for presymptomatic detection of cow-pox infection in mice using a novel combination of multivariate Bayesian statistics.

- Using assays for receptor activation, biocomputations and NMR we have learned important reasons for organ specific carcinogenesis in the rat. PhIP induces breast tumors because of its ability to mutate and enhance cell proliferation by activating the estrogen receptor while MeIQx, a stronger mutagen, does not because it inhibits the estrogen receptor activation.

- Showed that sperm of older men have increased frequencies of alkali-labile sites and/or DNA single-strand breaks, but not double-strand breaks, using the Comet assay.

- Determined that certain dietary intakes improved male fertility and decreased certain chromosomal defects in sperm. Specifically, we found that (a) antioxidant intake was associated with increased sperm motility; and (b) high folate intake is associated with lower levels of certain aneuploidies.

- Measured levels of metals in human sperm using particle induced x-ray emission (PIXE) and found significant differences in the relative abundances of several metals within sperm, but not in the semen fluid, with advancing male age.

- Showed that DNA adducts induced by the chemotherapeutic agent melphalan persist unrepaired in the sperm for several weeks and are converted into chromosomal aberrations in the fertilized egg.

- Conducted a field study in China to investigate the effects of occupational exposure to benzene on semen quality.

- Trained personnel from a biotechnology company in the use of mouse sperm FISH assay to detect chromosomal structural aberrations to be used for testing of chemicals of interest to the National Institute of Environmental Health Sciences.

- Developed protocols for isolating high quality RNA from small samples of tissues dissected from tissue sections using a laser capture system.

DNA Repair Mechanisms

- Developed a new mechanistic molecular model to account for the complex cellular phenotype of cells from the chromosome instability disorder, Fanconi anemia, which has cancer-predisposition and developmental defects.
- Constructed the first isogenic knockout mutant for homologous recombinational repair in an established mammalian line (hamster CHO cells).
- Demonstrated the importance of homologous recombination repair in classical S phase resistance of cycling mammalian cells exposed to ionizing radiation.
- Demonstrated the importance of the ATP binding motif (Walker A box) of the XRCC3 homologous recombination protein in regulating its binding to the Rad51C partner protein.

Reproductive and Developmental Biology

- Showed that sperm of older men have increased frequencies of alkali-labile sites and/or DNA single-strand breaks, but not double-strand breaks, using the Comet assay.
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- Trained personnel from a biotechnology company in the use of mouse sperm FISH assay to detect chromosomal structural aberrations to be used for testing of chemicals of interest to the National Institute of Environmental Health Sciences.

Molecular Biodosimetry

- Developed protocols for isolating high quality RNA from small samples of tissues dissected from tissue sections using a laser capture system.
- Developed protocols for performing whole-genome transcription and protein profiling in buccal cells.
- Developed techniques to process and analyze gene expression using limited cell samples derived from body fluids and laser microscopy dissected cells for future applications.
- Developed techniques for validation of gene transcript radiation biomarkers, identified by expression microarrays, in human cells using a real-time fluorescence based expression system.
- Identified potential radiation mRNA biomarkers for potential use in triage and field forward deployable applications.
- Instrumental in setting up the LLNL Microarray Center.

Carcinogenesis and Genetic Susceptibility

- Investigated gene expression changes with human consumption of green tea.

- Analyzed Singapore/Chinese meat samples for heterocyclic amines.

- Showed a complementary medicine actually increased mammary tumor incidence in rats.

- Showed that a food mutagen binds to the estrogen receptor, a finding that has implications for tumor tissue specificity.

- Detected a continuum of capacity to limit baseline DNA damage in untreated cells of cancer cases, controls, and hyper-normal individuals suggesting endogenous DNA damage could contribute to the identification of susceptible sub-groups.

Bioanalytical Technology

- Identified radiation dose markers in individual cells using time of flight secondary ion mass spectrometry.

- Devised new sample preparation method for biological cell surface analysis using mass spectrometry.

- Demonstrated ability to differentiate closely related cells based on secondary ion mass spectrometry.

- Designed and fabricated microfluid devices for cell-based assay of environmental toxic compounds.

- Developed mammalian cell-based assays that are integrated with the microfluid devices

Defense Biology Division

- Feasibility study of ultrasensitive electrochemical pathogen detection system resulting in patent application).

- Developed method to isolate strain-specific peptides derived from anthrax growth media for forensic analysis resulting in patent application in preparation.

- Discovered novel DNA oxidation products formed in human breast cancer cells (papers published in Journal of Biological Chemistry, Chemical Research in Toxicology and manuscript submitted to Bioorganic and Medicinal Chemistry Letters).

- Developed method to specifically label tyrosine and modified tyrosine residues in proteins in collaboration with the University of Washington (manuscript in preparation for submission to Nature Biotechnology).

- Used accelerator mass spectrometry to evaluate the pharmacokinetics of a novel antiviral drug candidate in collaboration with Merck Research Laboratories (published in Drug Metabolism and Disposition).

- Used accelerator mass spectrometry to evaluate the effectiveness of a novel computer-controlled drug delivery device in vivo in collaboration with MIT and Johns Hopkins (published in the Journal of Controlled Drug Release).

Genome Biology Division

- Participated in international efforts to sequence the chicken genome and to discover and validate genome-wide chicken SNP markers (two papers published in Nature).

- Completed the BAC-based sequence of a gene-rich chicken microchromosome, GGA28, and traced evolutionary conservation and change in gene content, regulatory sequence structure, and chromosome organization in comparisons with human and mouse genomes.

- Discovered a link between the proper function of potassium channel gene, Kcnq1, and chronic gastritis and stomach cancer in a mutant mouse model (published in Human Molecular Genetics).

- Collaboratively developed new computational tools for comparative genomics, including the ECR browser and a multi-species comparative alignment tool, MULAN (two papers in Nucleic Acids Research and Genome Research, respectively).

- Completed analysis of human gene deserts, discovering significant structural and functional differences between evolutionarily stable and lineage-specific gene-sparse regions (published in Genome Research).

- Developed a catalog of human zinc-finger loci including genes encoding more than 150 new and uncharacterized transcription factor proteins.

- The I.M.A.G.E. Consortium continues to provide arrayed cDNA resources to the community through a network of distributors. We have arrayed over eight million cDNAs from seven species (~550,000 cDNAs in 2003), resulting in over 6.6 million single-pass ESTs and high quality full-insert sequences submitted to Genbank so far.

- As part of the Mammalian Gene Collection (MGC) project, we have re-arrayed nearly 105,000 (19,000 in 2004) predicted full-length genes and made them available to the research community. The full-length sequences have been submitted to Genbank. At this time 85% of human and 75% of mouse known genes have a full-length I.M.A.G.E. clone representative from this project.

Bioinformatics and Biostatistics Group

- Cover article in Genome Research on "Evolution and functional classification of vertebrate gene deserts."

- Created numerous tools for genome analysis, including Mulan, a tool for multiple-sequence local alignment and visualization to study gene function and evolution.

- Started a new center, the Livermore Microarray Center, which will be a new lab-wide resource for expression array construction and analysis.

- Analyzed expression experiments for several environmental restoration-related microbes

- Created a new system for managing and tracking the work flow within the I.M.A.G.E Consortium project.

- Initiated design and development of an improved system for quality assurance of I.M.A.G.E. Consortium resources.

- Released two new builds of IMAGENE (http://image.llnl.gov).

- Designed and implemented an extensive new database system for Biosciences personnel management.

- Provided extensive bioinformatics and statistical expertise to numerous programs and projects within the Biosciences Program and the Joint Genome Institute's Production Genomics Facility in Walnut Creek.

2.0 Program Organization, Facilities and Resources

Organizational Structure

The Biosciences Directorate is organized around five research divisions and operational staff as shown in Figure 1b. The research divisions are:

Genome Biology Division
Defense Biology Division
Biomedical Division
Microbial Systems Division
Biotechnology Division

The Microbial Systems Division addresses a new area of growth for the Directorate tied closely to the goals of the DoE Office of Science Genomes-to-Life Program. With the support of the Laboratory, we are actively recruiting researchers and scientific leaders to develop this expertise in support of the DoE/OBER objectives.

The Biotechnology Division is also newly formed to address the Directorate's expanding suite of technology development needs and opportunities. The Biomedical Division has been newly organized as a consolidation of the Directorate's research activities of relevance to human health.

Directly supporting the Associate Director for Biosciences is a top-level management team consisting of a Principal Deputy Associate Director (PDAD), Al Ramponi, and a Deputy Associate Director for Operations (DADO), Sarah Wenning. Also reporting directly to the Associate Director is an Assurance Manager, Steve Mahler. Reporting to the PDAD is a team of three Program Development Leaders, two of which have so far been named: Elbert Branscomb (acting) for Genomes-to-Life and Ken Turteltaub for Defense Biology. Reporting to Sarah Wenning is a team of Operations Management leaders. Alan Casamajor acts as the Associate Director Facility Manager (ADFM). Patsy Gilbert serves as the ES&H Operations Manager and Select Agent Facility Manager to ensure the highest levels of safety and compliance with regards to regulatory requirements. The Business Manager, Cynthia Gardner, is responsible for our business operations and finances.

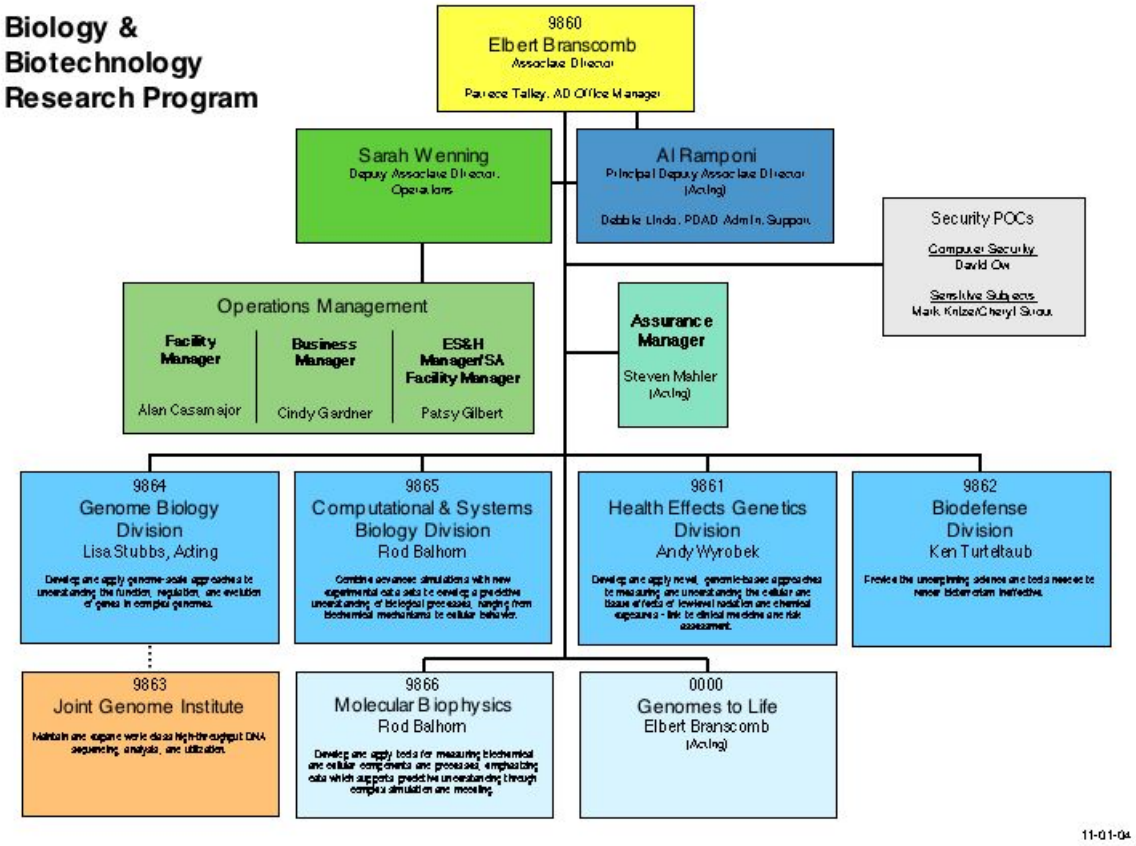

Figure 1a. Organization structure, November 2004.

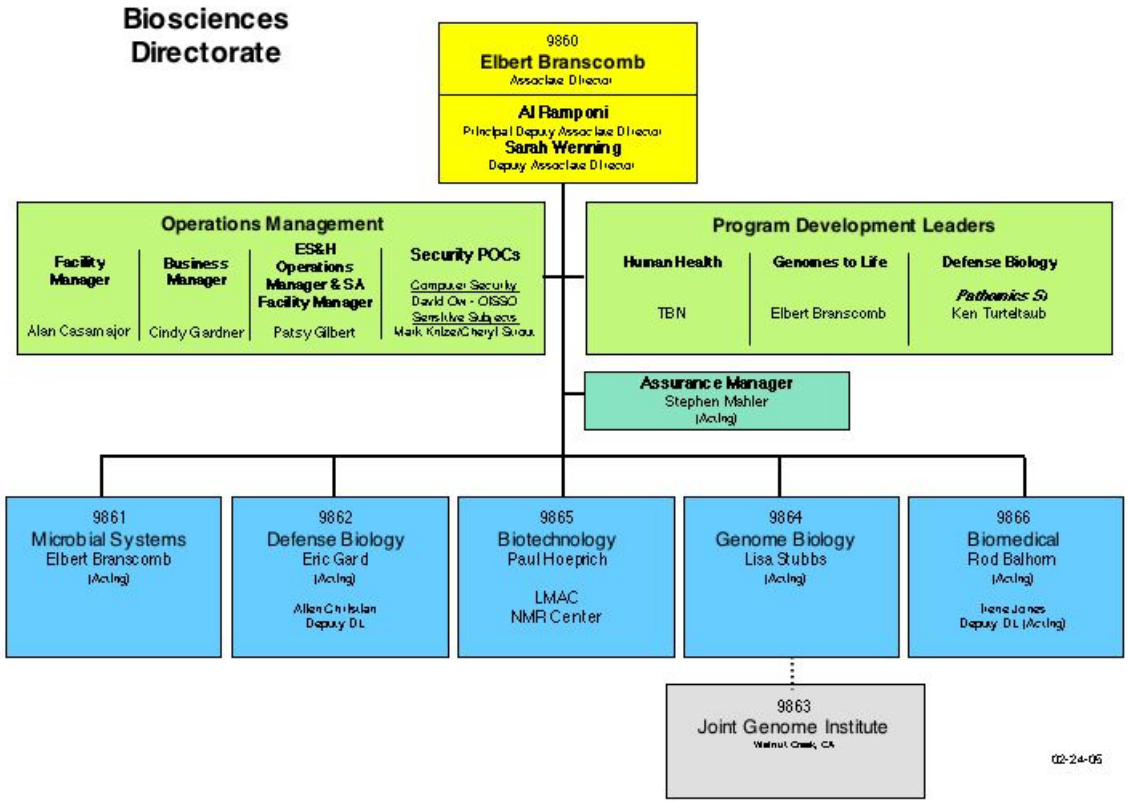

Figure 1b. Organization structure, February 2005.

Directorate Reviews and Advisory Committee

A variety of methods are used to assess the quality of our science and technology. At the project level, each federally funded effort is subject to a peer review process specific to its sponsor. For NIH and DOE, peer review is required for initial funding and for renewal. Every project is externally reviewed at least once every three years. At the Directorate level, we undergo a bi-annual internal review by the LLNL Director, several of the Associate Directors, other LLNL staff, and laboratory consultants. This review focuses on strategic planning, future scientific directions, intra-laboratory interactions, and workforce issues. Bioscience Directorate's Laboratory Directed Research is reviewed both internally to BIO and by an LLNL-wide committee. Our BIO Advisory Committee meets yearly to review the entire Directorate on a three-year cycle. The standing membership of the Advisory Committee is shown in Table 1. Ad-hoc reviewers are included each year as experts in the specific areas being reviewed.

Table 1. Directorate Review Committee

Biosciences Directorate Advisory Committee, 2004

Charles R. Cantor	Sequenom, Inc., Chair
Gilbert Chu	Stanford University Medical School
Fred F. Kadlubar	National Center for Toxicological Research
Hsing-Jien Kung	University of California, Davis Medical Center
Michael Levitt	Stanford University
Alex MacKerell	University of Maryland at Baltimore
Stephen A. Morse	Centers for Disease Control and Prevention
Frederick A. Murphy	University of California, Davis
Richard M. Myers	Stanford University School of Medicine
Kenneth H. Nealson	Jet Propulsion Laboratory
David A. Relman	Stanford University / Palo Alto Health Care
John A. Tainer	The Scripps Research Institute
Snorri Thorgeirsson	National Cancer Institute
James M. Tiedje	Michigan State University
Barbara J. Wold	California Institute of Technology
Peter Jahrling (ad hoc)	United States Army Medical Research Institute for Infectious Disease
Thomas Blumenthal (UC S&T Panel Representative)	University of Colorado School of Medicine

Lastly, the University of California, our contractor, conducts annual program reviews. The Science and Technology Council composed primarily of university faculty and appointed by the University President reviews the quality of science conducted at UC's three national laboratories. This Council meets with laboratory staff at least annually and receives written input from the Program Advisory Committee.

Budget Information

We draw upon several funding sources for our Directorate. While the Department of Energy traditionally has been our primary sponsor, in the past several years we have attracted a diverse assortment of funding sources. Funding amounts for each category are detailed in Table 2. The percentage funding by major funding source is shown in Figure 2. Appendix V details funding from DOE as well as non-DOE sources and from the LLNL Lab Directed Research and Development Program.

Table 2. Current funding sources

Sponsor	K$	Funding %
DOE, Office of Science/Office of Biological & Environmental Research	10,383	25.0
Department of Homeland Security	11,092	26.7
National Institutes of Health (Seven institutes)	8,632	20.8
LLNL Laboratory Directed Research and Development	8,620	20.8
LLNL General and Administrative	187	0.5
Army	211	0.5
Federal, Other	31	0.1
University of California, Berkeley	329	0.8
University of California, Breast Cancer Research Program	741	1.8
Nonfederal, Other	133	0.3
Lawrence Berkeley National Laboratory	266	0.6
University of California, Los Angeles	693	1.7
University of California, Davis	202	0.5
TOTAL	**41,519**	**100.0**

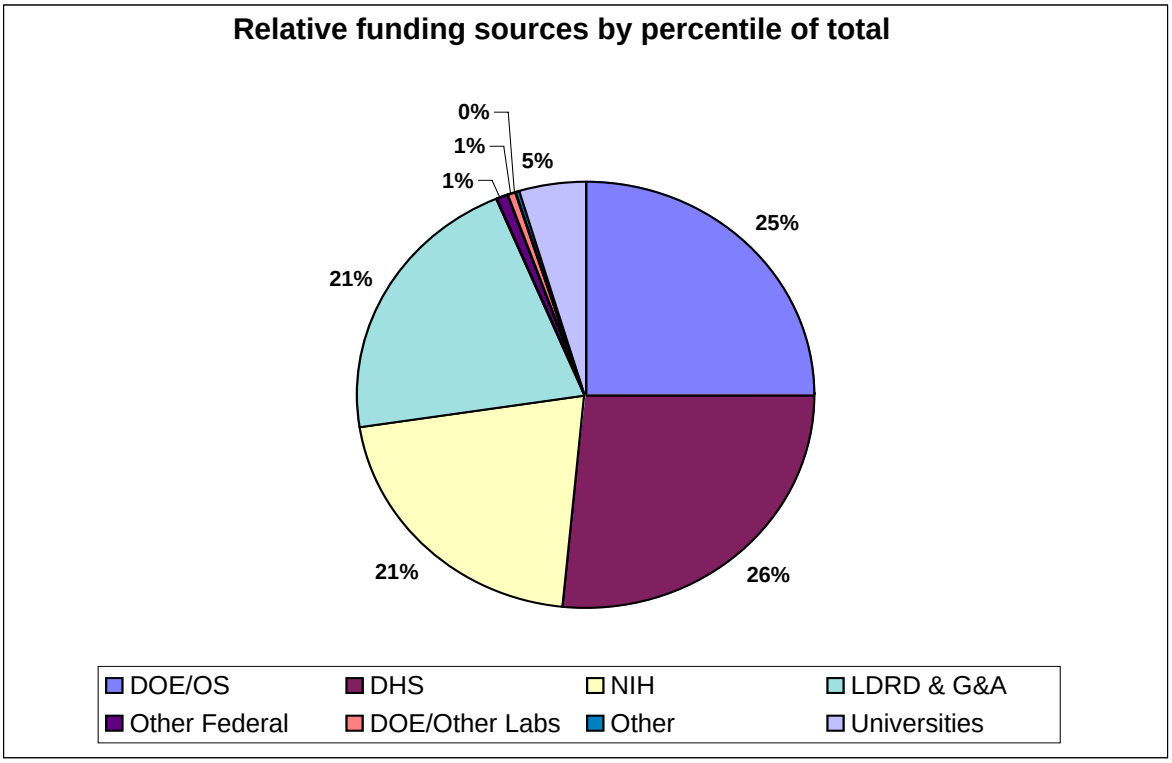

Figure 2. Relative funding sources by percentile of total.

Personnel Update

We draw from a diverse talent pool and facilitate staff advancement through formal training and education programs, self-training, and mentoring. Table 3 presents a snapshot of Biosciences Directorate personnel by category. Additional information including a roster of principal investigators and their extramural activities is available in Appendices I and II.

Table 3. Staff by Category – October 2004

Personnel	FY 2003	FY 2004
Principal Investigators	28	32
Other Scientific Staff	110	110
Technicians	34	35
Post- Doctorates	26	30
Graduate Students	5	5
Undergraduates	30	30
Total Scientific Personnel	**234**	**242**
Professional Admin.	14	14
Clerical Admin. Support	17	18
Total Admin. Personnel	**31**	**32**
TOTAL	**265**	**274**

The Biosciences Directorate provides educational and professional training opportunities for their staff, as well as to a wide array of students and faculty. The research and work experiences are designed to enhance the quality of science education at all levels and encourages diversity among people pursuing careers in the biomedical sciences. The programs integrate students and faculty into ongoing research projects and provide opportunities for them to expand their scientific and technical knowledge. The programs encourage students to continue their education in science. The summer programs allow students and faculty to extend and refresh their training and knowledge of areas of current research, and hence increase their ability to encourage students to pursue careers in science.

Internships (high-school through graduate level)

Scholar Employment Program: Offers high school through graduate-level students, teachers, and faculty the opportunity to engage in practical research experience to further their educational, research or teaching goals. Assignments are typically for:

* Summer – Full time during the summer academic break
* Co-op – Full time during the academic semester/quarter
* Year-round – Part-time during the academic year and full-time during the academic breaks

Summer Internship Program: This program targets recent graduates and graduate students pursing scientific studies who are majoring in science, math, engineering, or technical fields involving biology, chemistry, computer science, or physics.

Fellowships

Student Employee Graduate Research Fellowship Program: Graduate students currently at a UC campus who have completed their core courses and any relevant comprehensive examination in order to pursue research leading to a Ph.D degree may apply for a graduate fellowship through this program. The student, the university thesis advisor, and the Laboratory technical mentor as a team will jointly plan and execute the student's thesis research program.

Lawrence Fellowship: The purpose of this prestigious postdoctoral fellowship is to pursue cutting-edge science and stimulate cross-fertilization of ideas. The fellows will have freedom to pursue world-class research with ample resources to support their efforts. This fellowship has a three-year term. After this period, the fellows may consider any career options, including staying at the Laboratory. Typically, two to four fellowships are awarded each year.

Teacher development

Biotechnology Research Academy: This program is designed to give teachers experience in promoting and conducting research in biotechnology with their students. Teachers will develop knowledge, methods, and skills to be used in standards-based instruction when they return to their own classrooms..

Edward Teller Science & Technology Education Symposium: The goal of the symposium is to provide high school and community college science educators the opportunity to explore ongoing research at the Lawrence Livermore National Laboratory in chemistry, biology, environmental science, nuclear chemistry, biophotonics and astrophysics/fusion. Participants will tour state-of-the-art research laboratories, talk with researchers about their work, and attend "hands-on" workshops.

Student enrichment programs

Expanding Your Horizons: These conferences are one-day conferences for young women, grades 6-12, that are designed to encourage them to consider careers in math and science related fields.

Science on Saturday: This program is a seven-week series of free lectures and demonstrations intended for students 6th grade through high school. The topics are selected from the forefront of science and technology research in a variety of disciplines.

Tri-Valley Science and Engineering Fair: TVSEF is a science project competition for students grades 7-12 from schools within the areas of Danville, Dublin, Livermore,

Pleasanton, San Ramon, and Sunol. The fair is affiliated with the Intel International Science and Engineering Fair.

For more information on any of these programs, please visit http://www.llnl.gov/llnl/education/.

Facilities

The major building housing the Biosciences Directorate is a 68,000 gross square foot, one-story structure containing 55 laboratories, 83 offices, auditorium, conference rooms stock room, and administrative offices. Other laboratories and offices are located in fourteen nearby buildings and trailers, including special facilities for housing small animals, laboratories for handling virulent pathogens, a radiation exposure facility (a sealed 4,000 Ci ^{137}Cs source), an analytical chemistry laboratory, x-ray diffraction systems, and a high-level carcinogen laboratory (see Table 4). Just coming on line is a biosafety level 3 laboratory for handling especially virulent pathogens. This facility is owned by the NAI Directorate and will be operated by BIO under an memorandum of understanding.

BIO is projected to grow at a rate of 5% per year for the next several years and to develop new areas of research involving work with pathogenic microbes. B-368 will provide new microbial research capability. T-3629 (approximately 1,500 SF) was returned to the Institution for demolition. To replace it, the former BIO Library in T-3649 was converted into 17 offices plus a conference room. To support continuing growth, BIO is negotiating with the Institution to take over T-3725, a large modular facility to the north of the BIO campus.

Table 4. BIO Facilities

Facility	Use	Office (Sq.ft.)	Laboratory (Sq.ft.)
Bldg. 361	laboratory & office	12,382	24,863
Bldg. 362	laboratory & office	633	1,937
Bldg. 363	laboratory	0	1,119
Bldg. 364	animal care & laboratory	0	7,057
Bldg. 365	laboratory & office	188	2,969
Bldg. 366	laboratory & office	344	1,601
Bldg. 368	laboratory*	0	640
Bldg. 367	office	305	0
Bldg. 373	storage	0	0
Bldg. 376	shop & office	90	0
Bldg. 377	laboratory & office	349	2,823
Trlr 3649	office	1,952	0
Trlr 3703	office	6,469	0
Ttlr 3751	office	1,708	0
Trlr 3775	office	1,071	0
Trlr 3777	office	4,610	0
	Totals:	**30,101**	**43,009**

*Operated by BIO for NAI

Maintenance reinvestment support from the Institution continues to indicate a strong support for the Program. In 2004, the ageing boiler in B-361 was replaced with two new systems, one to provide hot water for space heat and the second to provide steam for process heating. These new systems are much easier to maintain and provide a significant improvement in reliability. In 2005, the boiler and chiller in B-364 will be removed. The chiller will be replaced with a new unit. The boiler will be replaced with a steam line connecting to the B-361 boiler. Work is beginning on a 7-year project to replace the B-361 roof and much of the HVAC equipment located upon it. A number of small improvement projects in several facilities are also being funded in support of new research directions.

Compliance with Federal Regulations

Lawrence Livermore National Laboratory has a long-standing tradition of concern for the welfare of animals used in research, in the protection and confidentiality of human subjects, and in the careful use of biohazardous materials and biological organisms. Permanent institutional committees supported by the laboratory exist in each area. The Laboratory Director appoints the chair of each committee.

Since 1973, the Animal Care Facility has been in full compliance with the Animal Welfare Act, the U.S. Public Health Service, the National Institutes of Health, the Office of Laboratory Animal Welfare, and the University of California regulations governing the use of animals in research. In 1976 we established an active Institutional Animal Care and Use Committee (IACUC), currently chaired by Francesco Marchetti, whose membership includes representatives from our scientific staff and the local community. Since July 2004, the LLNL IACUC serves as the IACUC of record for the newly constituted University of California at Merced. Our Animal Welfare Assurance, which dictates the institutional compliance requirements for LLNL, was recently renewed and is effective until June 30, 2008. We are also fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International and we are in the process of renewing our accreditation for another three years.

The Institutional Review Board (IRB) was formed at LLNL in 1974 to provide institutional assurance that all research activities involving human participants were being conducted under internationally recognized ethical principles, in compliance with U.S. federal regulations, and in adherence to the policies of the U.S. Department of Energy, Department of Health and Human Services (DHHS), State of California, and University of California. The LLNL IRB has the responsibility to identify and review all human research activities conducted by or involving LLNL employees to assure that the research is justifiable, and that all human participants are protected from unnecessary harms and risks. The LLNL IRB operates under general provisions of the Code of Federal Regulations, 10 CFR Part 745, revised June 1991, and holds a Federalwide Assurance (FWA #00004274) with DHHS, Office for Human Research Protections. The FWA dictates the specific institutional requirements for maintaining federal compliance and remains in effect until February 26, 2006. The LLNL Director provides the administrative

support for the IRB office (http://www.llnl.gov/HumanSubjects) and the review of the individual research protocols is conducted by the IRB which consists of volunteer scientific and non-scientific experts, including institutional and Livermore community members. The LLNL IRB, through a Memorandum of Understanding with the University of California at Merced, is currently the IRB of Record for that University. John P. Knezovich is the manager of the IRB office and chair of the IRB (knezovich1@llnl.gov) and Ann-Marie B. Dake is the IRB Administrator (dake1@llnl.gov).

The Institutional Biosafety Committee (IBC) was established at Lawrence Livermore National Laboratory in 1991 to ensure compliance with regulations concerning work with recombinant DNA or human, animal, and plant pathogens. These regulations include the NIH Guidelines for Research Involving Recombinant DNA Molecules, April 2002, and the CDC-NIH, Biosafety in Microbiological and Biomedical Laboratories, 1999 (Revision 4). All work in the cited subject areas must be approved by the IBC prior to initiation. A highly cross-referenced web site (http://ibc.llnl.gov) is available to help staff to understand and meet the Committee's requirements. The Committee consists of scientists, physicians, lawyers, and community members, and is co-chaired by Patsy Gilbert and Rod Balhorn.

3.0 Appendices

Appendix I: Biosciences Directorate PhD Staff and Project Managers

Principal Investigators / Project Managers

Ascher, Michael S.	MD	Harvard University, Boston, MA	Medicine
Balhorn, Rodney L.	PhD	University of Iowa, Iowa City	Biochemistry/Chemistry
Barsky, Daniel	PhD	University of Illinois, Urbana	Biophysics
Beernink, Peter	PhD	Boston University, Boston, MA	Biology
Branscomb, Elbert	PhD	Syracuse University, Syracuse, NY	Theoretical Physics
Chain, Patrick	MS	McMaster Univ., Ontario, Canada	Molecular Biology
Christian, Allen	PhD	Colorado State University, Ft. Collins	Chemical Engineering
Coleman, Matt	PhD	Boston University, Boston, MA	Molecular Biology
Cosman, Monique	PhD	New York University, New York	Physical Chemistry
Detter, John	PhD	University of Florida, Gainesville	Molecular Biology
Felton, James S.	PhD	State Univ. of New York, Buffalo	Molecular Biology; Zoology
Fidelis, Krzysztof A.	PhD	University of Oklahoma, Tulsa	Physical Chemistry
Garcia, Emilio	PhD	University of California, Davis	Microbiology
Gardner, Shea *	PhD	University of California, Davis	Ecology
Gordon, Laurie	BS	Graceland College, Lamoni, IA	Biology
Henderson, Paul	PhD	Georgia Inst. of Technology, Atlanta	Chemistry
Jones, Irene	PhD	University of Illinois, Urbana	Cell Biology
Kale, Patricia *	BS	University of California, Berkeley	Electrical Engineering/ Computer Science
Knize, Mark	BA	Calif. State Univ. Stanislaus, Turlock	Biology
Krishnan, Viswanathan	PhD	Indian Inst. of Science, Bangalore	Physics
Kryshtafovych, Andriy	PhD	Nat'l Academy of Science, Ukraine	Appl. Math. & Mechanics
Kulp, Kris	PhD	University of California, Davis	Pharmacology
Langlois, Richard G.	PhD	Columbia Univ, New York; UCBerkeley	Chemistry
Lightstone, Felice	PhD	Univ. of California, Santa Barbara	Chemistry
Liu, Nan	PhD	St. Andrews University, UK	Medicine
Loots, Gabriela	PhD	University of California, Berkeley	Microbiology
Lowe, Xiu	MS	California State Univ., Hayward	Molecular Biology
Lu, Xiaochen	MA	Tianjin Medical University, China	Pathology
Lucas, Susan	BA	Oregon State University, Corvallis	Biology
Manohar, Chitra	PhD	Indian Inst. of Science, Bangalore	Molecular Biology
Marchetti, Francesco	PhD	University of Rome, Italy	Biological Sciences
McCutchen-Maloney, S.	PhD	Texas A&M Univ., College Station	Chemistry
Mendelsohn, Mortimer L.	MD/ PhD	Harvard Medical School, Boston MA	Biophysics
Messenger, Sharon	PhD	University of Texas, Austin	Zoology
Moore, Dan	PhD	University of California, Berkeley	Statistics
Naraghi-Arani, Pejman	PhD	University of California, Davis	Plant Pathology
Nelson, David O. *	PhD	University of California, Berkeley	Statistics
Ovcharenko, Ivan *	PhD	Institute of Chemical Kinetics and Combustion, Novosibirsk, Russia	Physics/Mathematics
Ow, David *	MS	Calif. State Univ. Stanislaus, Turlock	Computer Science
Prange, Christa	BS	St. Mary's College, Moraga, CA	Biology

Radnedge, Lyndsay	PhD	University of London, UK	Bacteriology
Ramponi, Albert	PhD	University of Wisconsin, Madison	Chemistry
Rigl, Charles T. (Ted)	PhD	Georgia State University	Biochemistry
Rupp, Bernhard	PhD	University of Vienna, Austria	Chemistry
Segelke, Brent	PhD	University of California, San Diego	Chemistry
Simoes de Carvalho-Kavanagh, Marianne	PhD	University of California, Santa Barbara	Biology
Stubbs, Lisa J.	PhD	University of California, San Diego	Molecular Biology
Thelen, Michael P.	PhD	Cambridge University, England	Biochemistry
Thompson, Lawrence	PhD	Univ. Texas Health Center, Houston	Biophysics
Turteltaub, Kenneth	PhD	Iowa State University, Ames	Toxicology
Vitalis, Elizabeth	PhD	University of California, San Francisco	Biomedicine
Wyrobek, Andrew J.	PhD	University of Toronto, Canada	Medical Biophysics
Zhou, Carol *	PhD	University of Missouri, Columbia	Biological Sciences

* home organization is not BIO

Post-Doctoral Staff

Alegria-Hartman, Michelle	University of California, Davis	Genetics
Amer, Halima	Imperial College of Science and Technology, London	Chemistry
Bennion, Brian	University of Washington, Seattle	Biomedicine
Chromy, Brett	Northwestern University, Chicago, IL	Chemistry
Collette, Nicole	University of California, Davis	Genetics
Dover, Nir	Hebrew University, Jerusalem, Israel	Physiology/Biotechnology
Dugan, Lawrence	Colorado State Univ, Ft. Collins, CO	Radiobiology
Elso, Colleen	University of Melbourne, Australia	Genetics
Hah, Sang Soo	Seoul National University, Korea	Organic Chemistry
Hamilton, Aaron	University of California, Riverside	Biology
Hiddeson, Amy	Univ. of Pennsylvania, Philadelphia	Chemical Engineering
Hinz, John	University of Utah, Salt Lake City	Biology/Oncology
Huntley, Stuart	Washington State Univ, Pullman	Microbiology
Jeans, Christopher	Imperial College of Science, London	Biochemistry
Lau, Edmond	University of California, Santa Barbara	Chemistry
Laurence, Ted	University of California, Berkeley	Physics
Pesavento, Joseph	Baylor Coll. of Medicine, Houston, TX	Biochemistry
Redding, Kellie	Ohio State University, Columbus	Molecular Biology
Sawika, Dorota	Boston University, Boston, MA	Computational Chemistry
Shen, Nan	Harvard University, Boston, MA	Experimental Physics
Sulchek, Todd	Stanford University, Stanford, CA	Experimental Physics
Yamada, Nazumi	Univ. of N. Carolina, Chapel Hill, NC	Pathology
Zhang, Celia	Massay University, New Zealand	Biology/Genetics

***** home organization is not BIO

Appendix II: Staff Extramural Activities

Major Awards and Honors

B. Bennion	Department of Defense, Breast Cancer Concept Award, May 2004
P. Chain	International Society for Microbial Ecology Conference, Best Poster award
	BBRP Achievement Award
A. Christian	2004 R&D100 Award (siHybrid gene silencing)
	2004 Teller fellowship
J. Felton	Award of Merit, Second Term Comprehensive 10-Year Strategy for Cancer Control Program, Foundation for Promotion of Cancer Research, Japan, February 2004
P. Henderson	University of California, Davis, Cancer Center Annual Meeting Poster Session--Honorable Mention
K. Kulp	IDEA Award, California Breast Cancer Research Program
R. Langlois	BBRP Program Award for "Autonomous Pathogen Detection System"
	NAI Directorate Award for "most intriguing paper" from Chemical Abstracts Service
	Author and Point of Contact, R&D 100 Award for the Autonomous Pathogen Detection System

Invited lectures

R. Balhorn	10th Conference on Cancer Therapy with Antibodies and Immunoconjugates, Princeton, NJ, October 2004
P. Chain	Infectious Diseases and Immunity Colloquium, The University of Texas Medical Branch

A. Christian	RNAi Northern California Ataxia society annual meeting
	International Society of Analytical Cytometry, Current mechanisms of RNA interference, 2004
	BioSciences Forum, RNA interference, 2004
	British Consulate briefing, Gene Synthesis and Silencing, 2004
B. Chromy	Bay Area DIGE Users Group Meeting, San Francisco, CA, July 2004
	Cambridge Healthcare Institute Biodefense conference, Washington DC, August 2004
M. Coleman	Thirty-fifth Environmental Mutagen Society meeting, Pittsburgh, PA, October 2004
J. Felton	International Life Sciences Institute annual meeting symposium, Washington, DC, January 2004
	National Cancer Institute, Lab of Experimental Carcinogenesis Seminar, Bethesda, MD, January 2004
	Foundation for the Promotion of Cancer Research (NCRI), Tokyo, Japan, February 2004
	Chair, Agricultural Health Conference and Symposium, Washington, DC, February 2004
	Seminar, Linus Pauling Institute, Oregon State University, Corvallis, OR, May 2004
	Seminar, University of California, Davis, Cancer Center Retreat, Tahoe City, CA, May 2004
	Symposium speaker, National Cancer Institute special meeting on Molecular Epidemiology and Nutrition, Chicago, Il, September 2004
	Symposium speaker, International Nutrigenomics, Davis, CA, October 2004
	National Cancer Institute special meeting on Environmental Epidemiology and Risk, Philadelphia, PA, December 2004

E. Garcia	Invited to participate in Biosecurity Meeting organized by the Center for International Security, Stanford University and the McArthur Foundation, Moscow, Russia, December 2004
L. Gordon	Plant and Animal Genome XII Conference, San Diego, CA, Jan 2004
P. Henderson	Accelerator Mass Spectrometry Symposium for the American Chemical Society Division of Chemical Toxicology, Philadelphia, PA, August 2004
	Invited Speaker, University of California, Riverside, Environmental Toxicology Department
K. Kulp	"What's For Dinner" Jefferson National Laboratory Science Series, November 2004
R. Langlois	International Society of Analytical Cytology Congress XXIII, Montpellier, France, May 2004
	Seminar speaker, University of California Davis Cancer Center, Davis, CA, 2004
C. Manohar	Molecular Mechanisms and Cellular Consequences of Low-Dose Exposure to Ionizing Radiation in Mice and Human Cells, Applied Biosystems, Foster City, CA, March 2004
	Bi-National Conference on Radiological Responder Tools and Consequence Management Science and Technology Solutions Posters, LLNL, November 2004
S. McCutchen-Maloney	Proteomics in Applications in Therapeutic and Diagnostic Development, San Francisco, CA, June 2004
	International Association for Protein Structure Analysis and Proteomics, 15th Meeting of Methods in Protein Structure Analysis, Seattle, WA, August 2004
F. Marchetti	Assessing Germ-Cell Mutagenesis in the Post- Genome Era: A Celebration of the Legacy of William Lawson (Bill) Russell, The Jackson Laboratory, Bar Harbor, ME, September 2004

L. Stubbs	The Institute for Genomic Research Conference on Computational Genomics, Reston, VA, October 2004
T. Sulchek	American Society of Mechanical Engineers (ASME) Nanomechanics: Sensors and Actuators, Reno, NV, May 2004
L.H. Thompson	8th International Workshop on Radiation Damage to DNA, Banff, Alberta, Canada, May 2004
	Sixteenth Annual Fanconi Anemia Research Fund Scientific Symposium, Cambridge, MA, October 2004
	American Society for Microbiology Conference on DNA Repair and Mutagenesis: From Molecular Structure to Biological Consequences, Southampton, Bermuda, Nov 2004

Editorial Boards

R. Balhorn	*Molecular Reproduction and Development*
	International Journal of Biophysics
	Review of Scientific Instruments
A. Christian	*Expert Reviews In Proteomics*
M. Cosman	*NanoBiotechnology Journal*
J. Felton	*Mutation Research*
	Environmental and Molecular Mutagenesis
L. Thompson	*Molecular and Cellular Biology*
	Somatic Cell and Molecular Genetics
	DNA Repair

Societies (Major Roles)

J. Felton	International Conference on Environmental Mutagenesis Program Committee

P. Henderson	Member of the Executive Committee for the American Chemical Society Division of Chemical Toxicology

Advisory Committees and Grant Review Panels

R. Balhorn	Physical Biosciences Institute, LLNL
P. Chain	Proposal Study Panel Reviewer, Community Sequencing Program, Department of Energy/Joint Genome Institute
	Advisory Committee, LEK Consulting, LLC
M. Coleman	Industry/University Cooperative Research Program Biotechnology grant review panel
J. Felton	Chair, National Institutes of Health/Environmental Protection Agency Agricultural Health Study Advisory Board
	State of California Prop 65 Cancer Advisory Board
	University of California Toxic Substance Research &Training Program (UCTSR&TP) Executive Board
	National Cancer Institute Grants Subcommittee E (PO1 Oversight Panel)
	National Toxicology Program (NTP)/National Institute of Environmental Health Sciences (NIEHS) Report on Carcinogens (Heterocyclic Amines)- Testimony to Board of Scientific Councilors
	Executive Committee, University of California Davis Cancer Center
	National Cancer Institute intramural review of Laboratory of Human Carcinogenesis

E. Garcia	Invited participant: Fourth Annual Cooperative Biological Research Program Review Conference, St. Petersburg, Russia, July 2004
	Invited to develop and write a *Franscisella* comparative sequencing project proposal to be funded by NIAID
	Invited to develop and write a *Yersinia pestis* comparative sequencing project proposal to be funded by NIAID
R. Langlois	International Advisory Council for the Applied Ecology Research Laboratory
L. Radnedge	Grant review study section on Biodefense, National Institute of Allergy and Infectious Diseases (National Institutes of Health)
B. Segelke	LLNL Lab-Wide Laboratory Directed Research and Development (LDRD) review committee
L. Stubbs	Department of Energy Biological and Environmental Research Advisory Committee (BERAC)
	National Institutes of Health, Genomics, Computational Biology and Technology Study Section, Ad hoc reviewer
	University of California Davis Cancer Center Internal Advisory Committee
	Scientific Advisory Board, Oak Ridge National Laboratory Mouse Genetics User Facility

Meetings Organized

P. Chain	*Nostoc punctiforme* annotation and analysis, University of California, Davis, July 2004
K. Fidelis	Co-organizer, International Meeting on the Critical Assessment of Techniques for Protein Structure Prediction (CASP6), Gaeta, Italy, December 2004
P. Henderson	Accelerator Mass Spectrometry Symposium for the American Chemical Society Division of Chemical Toxicology, Philadelphia, PA, August 2004

A. Kryshtafovych	Co-organizer, International Meeting on the Critical Assessment of Techniques for Protein Structure Prediction (CASP6), Gaeta, Italy, December 2004
L. Stubbs	Advances in Genome Biology and Technology, Marco Island, FL, February 2004

Public Education and Outreach

R. Balhorn	Adjunct Professor, Department of Applied Science, University of California, Davis, Davis, CA
	Scientific Review Committee, Tri-Valley Science and Engineering Fair, March 2004
	Speaker, Teacher Research Academy, Edward Teller Education Center, December 2004
	Thesis supervisor and mentor for Ph.D. degree, Cheryl Dolan, University of California, Davis, Molecular and Cell Biology, completed June 2004
	Thesis supervisor and mentor for Ph.D. degree, Samantha Fore, University of California, Davis, Physics, ongoing
	Thesis supervisor and mentor for Ph.D. degree, Carol Hood, University of California, Davis, Immunology, ongoing
B. Bennion	Workshop leader, "Expanding Your Horizons" conference, Stockton, CA, October 2004
P. Chain	Judge, 8th Annual Tri-Valley Science and Engineering Fair, March 2004
	Mentor, National Science Foundation Integrative Graduate Education and Research Traineeship program
A. Christian	Science on Saturday lecture, Pleasanton, CA, February 2004
C. Elso	Point of Contact, LLNL Postdoc Networking Group

	Co-chair, Biosciences Directorate Postdoc Program Committee
	Speaker, Edward Teller Education Center Teacher training courses
	Workshop leader, "Expanding Your Horizons" conference
J. Hinz	Mystery Scientist, LLNL Discovery Science Saturday, Livermore, CA, July 2004
	Speaker, Teacher Research Academy, Edward Teller Education Center, July 2004
M. Kavanagh	Edward Teller Symposium, LLNL, Sept 2004
K. Kulp	Project mentor, High School Science Fair, December 2004
C. Manohar	Workshop leader, Expanding Your Horizons in Science and Mathematics, Stockton, CA, October 2004
	Judge, 8th Annual Tri-Valley Science and Engineering Fair, March 2004
	LLNL Microarray Center Group Tours for High School students throughout 2004.
S. McCutchen-Maloney	Speaker, Department of Homeland Security Summer Intern Lecture Series, 2004
	Panel member, LLNL Institutional Education Committee for "Women in Science: Career Challenges", 2004
L. Stubbs	Student lecture and interaction, University of California Davis Genetics Graduate Group, January 2004.
	Invited lecturer, graduate student seminar series, Hubrecht Laboratories, University of Utrecht, Utrecht, The Netherlands, March 2004
	Adjunct Professor, University of Tennessee, Knoxville/Oak Ridge National Laboratory, Department of Genome Sciences

T. Sulchek	Consultant, characterization and optimization for intraocular lenses for cataract surgery in India, Project Impact
	Project mentor, MEMs design class ME342, Stanford University, Palo Alto, CA

Appendix III: Publications and other information transfer

Biosciences Directorate utilizes many different methods to transfer information to the public, including those listed in the table below. Data for 2003 is included for comparison. Publication information constitutes the remainder of this appendix.

Information type	CY2003	CY2004
Peer-reviewed publications	66	71
Book chapters	4	1
Abstracts and posters	n/a	100
Patents issued	4	3
Patents pending	5	8
Invention disclosures	8	9
Income from royalties and licensing (FY04)	~$190,000	~$198,984
cDNA clones made available	~832,000	~550,000

Manuscripts and book chapters published (2004)

Bennett, L. M., Montgomery, J. L., Steinberg, S. M., and Kulp, K. S. (2004). Flor-Essence herbal tonic does not inhibit mammary tumor development in Sprague Dawley rats. Breast Cancer Res Treat 88, 87-93.

Camarero, J. A., Kwon, Y., and Coleman, M. A. (2004). Chemoselective attachment of biologically active proteins to surfaces by expressed protein ligation and its application for "protein chip" fabrication. J Am Chem Soc 126, 14730-14731.

Chain, P. S., Carniel, E., Larimer, F. W., Lamerdin, J., Stoutland, P. O., Regala, W. M., Georgescu, A. M., Vergez, L. M., Land, M. L., Motin, V. L., et al. (2004). Insights into the evolution of *Yersinia pestis* through whole-genome comparison with *Yersinia pseudotuberculosis*. Proc Natl Acad Sci U S A 101, 13826-13831.

Chan, S., Segelke, B., Lekin, T., Krupka, H., Cho, U. S., Kim, M. Y., So, M., Kim, C. Y., Naranjo, C. M., Rogers, Y. C., et al. (2004). Crystal structure of the *Mycobacterium tuberculosis* dUTPase: insights into the catalytic mechanism. J Mol Biol 341, 503-517.

Chepanoske, C. L., Brown, K., Turteltaub, K. W., and Dingley, K. H. (2004). Characterization of a peptide adduct formed by N-acetoxy-2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), a reactive intermediate of the food carcinogen PhIP. Food Chem Toxicol 42, 1367-1372.

Chiarappa-Zucca, M. L., Finkel, R. C., Martinelli, R. E., McAninch, J. E., Nelson, D. O., and Turteltaub, K. W. (2004). Measurement of beryllium in biological samples by accelerator mass spectrometry: applications for studying chronic beryllium disease. Chem Res Toxicol 17, 1614-1620.

Chromy, B. A., Gonzales, A. D., Perkins, J., Choi, M. W., Corzett, M. H., Chang, B. C., Corzett, C. H., and McCutchen-Maloney, S. L. (2004). Proteomic analysis of human serum by two-dimensional differential gel electrophoresis after depletion of high-abundant proteins. J Proteome Res 3, 1120-1127.

Chromy, B. A., Perkins, J., Heidbrink, J. L., Gonzales, A. D., Murphy, G. A., Fitch, J. P., and McCutchen-Maloney, S. L. (2004). Proteomic characterization of host response to *Yersinia pestis* and near neighbors. Biochem Biophys Res Commun 320, 474-479.

Coleman, M. A., Lao, V. H., Segelke, B. W., and Beernink, P. T. (2004). High-throughput, fluorescence-based screening for soluble protein expression. J Proteome Res 3, 1024-1032.

Cosman, M., Krishnan, V. V. and Balhorn, R. (2004). Application of NMR methods to identify detection reagents for use in the development of robust nanosensors, in Protein Nanotechnology: Protocols, Instrumentation and Applications (Tuan Vo-Dinh, Editor) Humana Press, Totowa, NJ, 141-163.

Cupid, B. C., Lightfoot, T. J., Russell, D., Gant, S. J., Turner, P. C., Dingley, K. H., Curtis, K. D., Leveson, S. H., Turteltaub, K. W., and Garner, R. C. (2004). The formation of AFB(1)-macromolecular adducts in rats and humans at dietary levels of exposure. Food Chem Toxicol 42, 559-569.

Dingley, K. H., Ubick, E. A., Vogel, J. S., and Haack, K. W. (2004). DNA isolation and sample preparation for quantification of adduct levels by accelerator mass spectrometry. Methods Mol Biol 291, 21-28.

Elso, C. M., Lu, X., Culiat, C. T., Rutledge, J. C., Cacheiro, N. L., Generoso, W. M., and Stubbs, L. J. (2004). Heightened susceptibility to chronic gastritis, hyperplasia and metaplasia in Kcnq1 mutant mice. Hum Mol Genet 13, 2813-2821.

Felton, J. S., Knize, M. G., Bennett, L. M., Malfatti, M. A., Colvin, M. E., and Kulp, K. S. (2004). Impact of environmental exposures on the mutagenicity/carcinogenicity of heterocyclic amines. Toxicology 198, 135-145.

Fergenson, D. P., Pitesky, M. E., Tobias, H. J., Steele, P. T., Czerwieniec, G. A., Russell, S. C., Lebrilla, C. B., Horn, J. M., Coffee, K. R., Srivastava, A., et al. (2004). Reagentless detection and classification of individual bioaerosol particles in seconds. Anal Chem 76, 373-378.

Forde, C. E., Rocco, J. M., Fitch, J. P., and McCutchen-Maloney, S. L. (2004). Real-time characterization of virulence factor expression in *Yersinia pestis* using a GFP reporter system. Biochem Biophys Res Commun 324, 795-800.

Friddle, R. W., Klare, J. E., Martin, S. S., Corzett, M., Balhorn, R., Baldwin, E. P., Baskin, R. J., and Noy, A. (2004). Mechanism of DNA compaction by yeast mitochondrial protein Abf2p. Biophys J 86, 1632-1639.

Gerhard, D. S., Wagner, L., Feingold, E. A., Shenmen, C. M., Grouse, L. H., Schuler, G., Klein, S. L., Old, S., Rasooly, R., Good, P., et al. (2004). The status, quality, and expansion of the NIH full-length cDNA project: the Mammalian Gene Collection (MGC). Genome Res 14, 2121-2127.

Gilbert, M., Edwards, T. C., and Albala, J. S. (2004). Protein expression arrays for proteomics. Methods Mol Biol 264, 15-23.

Grimwood, J., Gordon, L. A., Olsen, A., Terry, A., Schmutz, J., Lamerdin, J., Hellsten, U., Goodstein, D., Couronne, O., Tran-Gyamfi, M., et al. (2004). The DNA sequence and biology of human chromosome 19. Nature 428, 529-535.

Hillier, L. W., Miller, W., Birney, E., Warren, W., Hardison, R. C., Ponting, C. P., Bork, P., Burt, D. W., Groenen, M. A., Delany, M. E., et al. (2004). Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. Nature 432, 695-716.

Kantardjieff, K., and Rupp, B. (2004). Structural bioinformatic approaches to the discovery of new antimycobacterial drugs. Curr Pharm Des 10, 3195-3211.

Kantardjieff, K. A., Jamshidian, M., and Rupp, B. (2004). Distributions of pI versus pH provide prior information for the design of crystallization screening experiments: response to comment on 'Protein isoelectric point as a predictor for increased crystallization screening efficiency'. Bioinformatics 20, 2171-2174.

Kantardjieff, K. A., Kim, C. Y., Naranjo, C., Waldo, G. S., Lekin, T., Segelke, B. W., Zemla, A., Park, M. S., Terwilliger, T. C., and Rupp, B. (2004). *Mycobacterium tuberculosis* RmlC epimerase (Rv3465): a promising drug-target structure in the rhamnose pathway. Acta Crystallogr D Biol Crystallogr 60, 895-902.

Kantardjieff, K. A., and Rupp, B. (2004). Protein isoelectric point as a predictor for increased crystallization screening efficiency. Bioinformatics 20, 2162-2168.

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Langlois, R. G., Trebes, J. E., Dalmasso, E. A., Ying, Y., Davies, R. W., Curzi, M. P., Colston, B. W., Jr., Turteltaub, K. W., Perkins, J., Chromy, B. A., et al. (2004). Serum protein profile alterations in hemodialysis patients. Am J Nephrol 24, 268-274.

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Appendix IV: Patents and Invention Disclosures

Patents Issued – 2004		
Inventors	Title	Patent No.
Ward Small IV, Peter Celliers	Single-Fiber Multi-Color Pyrometry	6682216
Stefan P. Swierkowski	Microchannel Cross Load Array with Dense Parallel Input	6716328
Allen T. Christian, Melissa S. Pattee, Cristina M. Attix, James D. Tucker	Rolling Circle Amplification Detection of RNA and DNA	6783943

Patents Pending – 2004		
Inventors	Title	Tracking No.
Peter T. Beernink	High Throughput Protein Production Screening	11157
Adam T. Zemla	Local-Global Alignment for Finding 3D Similarities in Protein Structures	11160
Paul Thomas Henderson	Light-Mediated Electrochemical Pathogen Detection System	11212
Robin R. Miles, Jackie J. Crawford, Allen T. Christian	Device for Locating Cells Analysis in Vertical Format	11324
Robin R. Miles, Jackie J. Crawford, Allen T. Christian	Device for Locating Cells and Aids for Cellular Analysis	11325
William A. Romine, Sharon J. Shields	Mass Spectral Data Integration and Reduction Algorithms	11340
Brent William Segelke, Timothy Lekin	Liquid Class Predictor for Liquid Handling of Complex Mixtures	11367
Allen T. Christian, Larry Dugan, Joel Bedford	Repetitive Sequence-free DNA Libraries	11465

Appendix IV: Patents and Invention Disclosures, cont'd

Invention Disclosures - 2004		
Inventors	Title	Tracking No.
Robin R. Miles, Jackie J. Crawford, Allen T. Christian	Device for Locating Cells Analysis in Vertical Format	11324
Robin R. Miles, Jackie J. Crawford, Allen T. Christian	Device for Locating Cells and Aids for Cellular Analysis	11325
Brent W. Segelke, Dominique G. Toppani, Timothy P. Lekin	Labware for High Throughput Free Interface Diffusion Crystallization	11335
William A. Romine, Sharon J. Shields	Mass Spectral Data Integration and Reduction Algorithms	11340
Brent William Segelke, Timothy Lekin	Liquid Class Predictor for Liquid Handling of Complex Mixtures	11367
Andrew Wyrobek, Matthew A. Coleman, David Nelson, James Tucker	Ionizing Radiation Transcript Panel for Biodosimetry	11383
Todd A. Sulchek, Siping R. Qiu	Molded Microfluidic Fluid Cell for Atomic Force Microscopy	11414
Allen T. Christian, Christine Hara	Multiple Displacement Amplification with Blocker DNA	11452
Mark G. Knize, Kristen Kulp, Kuangjen Wu	Sample Preparation Device for Biological Cells for Use With Imaging Mass Spectrometry	11466

Appendix V: Funding information (listed by sponsoring agency)

Work For Others:

Sponsor	Support (K$)	Principal Investigator	Objective
ARMY	127.6	K. Dingley	Determine structure of protein adducts of PhIP, develop an assay to measure them and use the assay to measure adduct levels in children
ARMY	83.0	B. Bennion	To develop and test a prediction tool for searching new compounds to treat breast cancer. These drugs would be used in tandem with current therapeutics
DEPARTMENT OF STATE	30.5	L. Thompson	Analyze mutations in the XPB gene in 8 hamster cell lines with abnormally high sensitivity to killing by UV-C radiation
HHS/CDCP	713.9	K. Fidelis	To support the continuing operation of the Critical Assessment of Protein Structure Prediction (CASP) process and to expand its infrastructure,
HHS/CNRR	2,533.7	K. Turteltaub	Develop and demonstrate biomedical applications of AMS
HHS/NIGMS	381.2	J. Kim	Understand the molecular mechanism that controls the imprinting of several genes and testing the potential involvement of the imprinted genes to human chromosome
HHS/NCI	17.8	I. Jones	Measure the level of DNA damage using an assay developed in BIO
HHS/NCI	2,555.8	J. Felton	Determine whether heterocyclic amine mutagens/carcinogens contribute to human cancer incidence

HHS/NIDDK	47.4	C. Prange	Provide clone rearraying, distribution and archival services to the sponsor organization, along with customized web-based data and process tracking tools to support the project
HHS/NCCRM	280.2	K. Kulp	Studying the interactions of Flor-Essence^{R} Tonic & PhIP
HHS/NCI	364.7	L. Thompson	To understand the molecular regulatory processes cells use to minimize genetic damage and genetic instability associated with reactive oxygen species arising from endogenous processes or ionizing radiation
HHS/NIAID	266.3	E. Garcia	Gain understanding as to how acute and highly lethal bacterial pathogen such as *Yersinia pestis* has evolved.
HHS/NCI	1,471.0	C. Prange	Isolate large numbers of full-length genes and make them publicly available.
Integrated Laboratory Systems	53.2	F. Marchetti	Train ILS scientists in scoring the mouse CT8 assay
University of South Florida	10.0	P. Chain	Determine the complete genomic sequence of the environmental microbe *Thiomicrospira crunogena*
University of Toronto	70.0	J. Albala	To generate high density protein biochips
Lawrence Berkeley National Laboratory	130.8	L. Thompson	To determine the molecular structures of human DNA repair proteins that participate in homologous recombination

Lawrence Berkeley National Laboratory	14.7	C. Manohar	
Lawrence Berkeley National Laboratory	120.0	L. Radnedge	Analyze the nucleotide sequence of the genomes of *Yersinia pestis* strains Antique and Nepal 516 for SNP discovery and validation
UC Los Angeles	692.6	B. Rupp	Carry out large scale crystallizations of proteins from *M. tuberculosis*
UC Berkeley	127.4	A. Wyrobek	nvestigate the relationship between aneuploidy sperm and aneuploidy at birth caused by parental age, diet, and smoking
UC Berkeley	202.0	K. Turteltaub	Address the effects of chemical dose on the absorption and the associated DNA and protein adduct levels for exposure to benzene and trichlorethylene, carcinogens found at superfund waste sites and common to the urban environment.
UC Davis	17.2	A. Christian	Develop a process for detecting DNA mutations in blood plasma to be used to detect recurrence of tumors in cancer patients following chemotherapy
UC Davis	161.5	R. Balhorn	To design and synthesize novel high affinity, small multivalent targeting and radioisotope carrier molecules (SHALs) that bind selectively to human lymphoma cells and provide fast clearance (lower dose to normal tissues) and rapid uptake and “near-permanent” binding to the cancer

UC Davis	23.1	K. Dingley	To establish the histological changes occurring in rat prostate following exposure to the prostate carcinogen PhIP
UC Breast Cancer Research Program	389.4	P. Henderson	Develop an assay for DNA-based biomarkers of breast cancer
UC Breast Cancer Research Program	167.8	K. Kulp	Determine if circulating tumor cells (CTCs) can be separated from peripheral blood samples and then identified using Time-of-Flight Secondary Mass Spectrometry
UC Breast Cancer Research Program	183.8	K. Kulp	Evaluation of essiac tea to prevent mammary tumors and improve our understanding of the impact of a complementary and alternative therapy
TOTAL	**11,236.6**		

DOE (Office of Biological & Environmental Research):

Support (K$)	Principal Investigator	Objective
475.0	L. Thompson	Understand the relative contributions of the individual DNA-damage response pathways to the recovery of mammalian cells from exposure to IR in the range of 0-1 Gy
24.0	M. Coleman	
757.0	A. Wyrobek	Provide mechanistic and molecular knowledge of the cellular response to low dose IR to help reduce the uncertainty in assessing health risks, and identify candidate genes for differential susceptibility to low-dose IR exposure
6,200.0	JGI	Make significant contribution to the international Human Genome Project
100.0	C. Prange	The I.M.A.G.E. Consortium will work to isolate large numbers of FL cDNA clones
400.0	L. Stubbs	Develop a mouse mutant resource to link human genes to health-related functions
500.0	L. Stubbs	Characterize a region of HSA19q13.4 containing imprinted genes, and study the evolutionary history by comparative analysis of genomic sequence from multiple species
1,569.2	L. Stubbs	Functional annotation of genes and regulatory sequences on HSA19
343.1	I. Ovcharenko	
15.0	E. Branscomb	GTL workshop
10,383.3	**TOTAL**	

Department of Homeland Security:

Support (K$)	Principal Investigator	Objective
152.0	R. Balhorn	Toxins
3,491.0	A. Christian	BioBriefcase
47.5	E. Garcia	BDAP
406.5	E. Garcia	Profiling Transcriptional Response
532.7	R. Balhorn	Next Generation Assays
1,462.0	S. McCutchen-Maloney	Human Assay Development
1,092.1	S. McCutchen-Maloney	Tailored Assays for Detection of Agro Terrorism
1,387.5	S. McCutchen-Maloney	Viral Genomics
1,937.6	S. McCutchen-Maloney	Host Pathogen Pathways
582.8	E. Garcia	Georgia Collaboration
11,091.7	**TOTAL**	

LLNL (Laboratory Directed Research and Development):

Support (K$)	Principal Investigator	Objective
189.3	P. Beernink	Assess the effects of mutations that cause human diseases using computational and experimental approaches
3,905.0	K. Turteltaub	Develop an approach rapidly leading to assays to detect protein and metabolite signatures
40.0	M. Thelen	Letant Collaboration
490.0	L. Stubbs	Establish new technologies for characterizing transcription factor (TF) proteins and their regulatory pathways
943.0	A. Christian	Develop and apply technical capabilities to create a new LLNL core competency to measure, manipulate and predictively model life at the level of individual cells

36.0	M. Corzett	Hollars Collaboration
410.0	K. Fidelis	Develop a new approach to identification of gene regulation mechanisms based on a supervised machine learning method
50.0	K. Fidelis	Develop a global database of theoretically derived protein structures
90.0	K. Kulp	Use Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) to characterize bacterial metabolites in individual bacteria
580.0	A. Wyrobek	Identify radiation response of mechanisms and molecular switches that enhance radioresistance.
492.2	G. Loots	Develop tools for determining the function of novel human genes
380.0	H. Beller	
90.0	S. Gardner	Develop tools to perform electronic PCR (ePCR). Build new computational methods to model the kinetic, thermodynamic and biological processes of PCR reactions.
176.5	C. Venclovas	
190.0	R. Balhorn	Determine whether light-emitting polymers can be used to detect pathogen DNA and various types of damaged DNA at concentrations that enable their use in clinical and biodefense applications
190.0	D. Barsky	Combined experimental and computational approach to investigate the interaction between DNA and a sliding-clamp protein.

112.0	P. Chain	Implement, test and experimentally validate the results of an algorithm for the genome-wide identification of candidate transcription factor binding sites in prokaryotes
75.0	P. Henderson	Development of a low-cost rapid pathogen DNA detection system that does not require expensive reagents and can be automated
66.0	J. Albala	
75.0	F. Marchetti	Evaluate the feasibility of (a) using a newly acquired laser-capture microdissection system to identify and isolate target cells from tissue sections using immuno- and histochemical labeling while retaining cellular messenger RNA and protein integrity and (b) develop small sample gene-transcript analysis protocols to define genome-wide expression profiles of isolated cells.
40.0	Sulchek	Develop a microfluidic liquid cell for in situ molecular imaging in aqueous solution using atomic force microscopy (AFM)
8,620.0	**TOTAL**	

LLNL (General and Administrative):

Support (K$)	**Principal Investigator**	**Objective**
187.0	M. Mendelsohn	Melanoma Investigation
187.0	**TOTAL**	